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Bacterial Growth in Expressed Breastmilk  
in Continuous Feeding Setups in the  
Neonatal Intensive Care Unit

by

Christene Marie Evanochko



A thesis submitted to the Faculty of Graduate Studies and  
Research in partial fulfilment of the requirements for the  
degree of Master of Nursing

Faculty of Nursing

Edmonton, Alberta

Fall 1995



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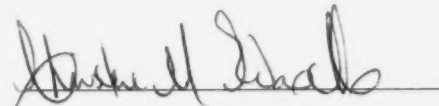
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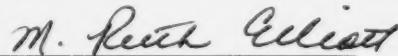
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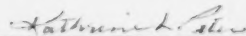
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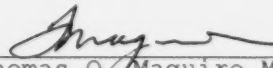
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Date: October 3, 1995

### Abstract

Premature and high-risk neonates who are unable to breastfeed in the Neonatal Intensive Care Unit (NICU) may receive their mother's milk through a continuous infusion method.

This study examined the effect of syringe change frequency of expressed breastmilk (EBM) on the amount of bacterial growth in a continuous feeding setup (CFSU). A factorial within-groups experimental design was used to compare bacterial growth in EBM delivered through CFSUs. Forty-five EBM samples were divided to infuse through two simulated CFSUs, one with hourly syringe changes (control) and one with four hourly syringe changes (experimental). Cultures from each EBM sample were drawn at baseline, one and four hours infusion in each CFSU, and post-study.

Quantitative culture analysis identified non-pathogenic (*Coagulase negative staphylococci*, *Viridans group streptococci*, *Diphtheroids*, *Non-pathogenic neisseria species*) and pathogenic bacteria (*Staphylococcus aureus*, *Yeast*, *Non-lactose fermenting coliforms*, *Lactose fermenting coliforms*) in the EBM samples. Repeated measures ANOVA and chi-square analysis showed no significant difference in the bacterial growth between the control and experimental CFSU over a four hour period. An independent measures t-test revealed a statistically significant difference ( $p < .05$ ) on the amount of *Coagulase*

*negative staphylococci* growth in EBM samples collected in the NICU as compared to those collected in the home.

This study analyzed the efficacy of current nursing practice and supports infusing EBM over a four hour period without an increased risk of bacterial growth. Study findings represent a potential savings of \$10,000.00 per year on syringes along with an estimated ten minutes per hour of nursing time or 4 hours per day (approximately \$31,000.00 per year of salary savings).

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## CHAPTER ONE

### INTRODUCTION

For many years, nursing practice has been based mainly on routine and tradition, not always backed by scientifically conducted evaluation. In order to justify resource allocation and budget cuts, which are major issues faced daily in our health care system, practice-related routines should be evaluated through research to determine effectiveness. Not only can practice be improved as a result of research findings, but also decisions relating to the issues of nursing time and cost effectiveness can be addressed (Alexander & Kelly, 1991; Barry & Miller, 1988; Dodd & Froman, 1991; Hu, Kaltreider & Igou, 1990; McMurdo, Davey, Elder, Miller, Old, & Malek, 1992).

The following are examples of nursing research studies which have changed practice and resulted in cost-savings. Fox (1990) evaluated the accuracy of diaper weight measurements in the Neonatal Intensive Care Unit (NICU) through a simulation research study. Based on the results of her research, the method and accuracy of diaper weighing has been adopted into current nursing practice. Dodd and Froman (1991) evaluated the bacterial growth in expressed breastmilk (EBM) and formula. Based on their results, the frequency that nursing staff changed feeding setups decreased from every 4 hours to every 8 hours. This study

resulted in both cost and nursing time savings.

An example of current practice needing evaluation is the method of administering EBM in a continuous feeding setup (CFSU) to premature and high-risk neonates in the NICU. Nurses in our NICU were replacing the syringe of EBM in a CFSU hourly, due to concerns about increased bacterial contamination to the vulnerable premature and high-risk neonate. Not only can bacterial contamination result in neonatal sepsis, but also nursing time and related costs spent changing syringes of EBM are factors to be considered (Dodd & Froman, 1991).

This nursing practice-based research study addressed the effect of frequency of syringe changes within a CFSU in the NICU on bacterial contamination of EBM. A simulated factorial within-groups experimental design conducted in the NICU setting was utilized to address this research question. EBM samples were obtained for this study from forty-five healthy volunteer breastfeeding mothers of term infants ( $\geq$  37 weeks gestation or post-conceptual age).

#### **Purpose**

The purpose of this study was to examine the effect of syringe change frequency on bacterial growth in EBM within a CFSU in the NICU. One hour syringe changes were compared with four hour syringe changes in CFSUs. The rationale for choosing these frequencies was twofold: current NICU

practice and recommendations of the American Pediatric Society and the American College of Obstetricians and Gynecologists (1992) that EBM is safe to infuse in a CFSU at room temperature for four hours.

#### **Research Question**

Does the frequency of syringe changes of EBM (one hourly compared to four hourly) affect the amount of bacterial growth in continuous feeding setups in the NICU?

#### **Hypothesis**

There will be significantly lower bacterial colony counts ( $< 10^7$  cfu/L) in a CFSU in which syringes of EBM are replaced hourly as compared to a CFSU in which syringes of EBM are replaced every four hours.

#### **Definition of Terms**

##### **Expressed Breastmilk**

Expressed breastmilk is defined as human milk which has been expressed into a sterile glass bottle using the Medela or Medela Lactina electric breastpump system.

##### **Continuous Feeding Setup**

Continuous feeding setup(CFSU) is defined as the feeding system which is used in the NICU to deliver breastmilk to neonates who cannot breastfeed and require their feeds to be delivered at a continuous set infusion rate. This system includes the following: a 10cc Becton-Dickinson syringe with EBM connected to a 2 ml extension

tubing and delivered to the neonate's nasogastric feeding tube via a Medfusion #2001 infusion pump. For the purpose of this research study, human subjects were not used and the CFSU delivered the EBM into a sterile collection bag via a #8-Fr nasogastric feeding tube simulating a CFSU with a live infant in the NICU (see Figure 1).

#### **Significance of the Study**

Addressing the issue of bacterial growth in EBM in CFSU will assist neonatal nurses in determining how frequently syringes of EBM should be replaced in a feeding setup without posing increased risk to the neonate. The results of this study will contribute to knowledge about the efficacy of current nursing practice in the NICU with the goal to improve outcomes for this highly vulnerable population. Cost-savings related to the amount of syringes used in a 24 hour period will be addressed as well as the amount of nursing time spent replacing these syringes of EBM.

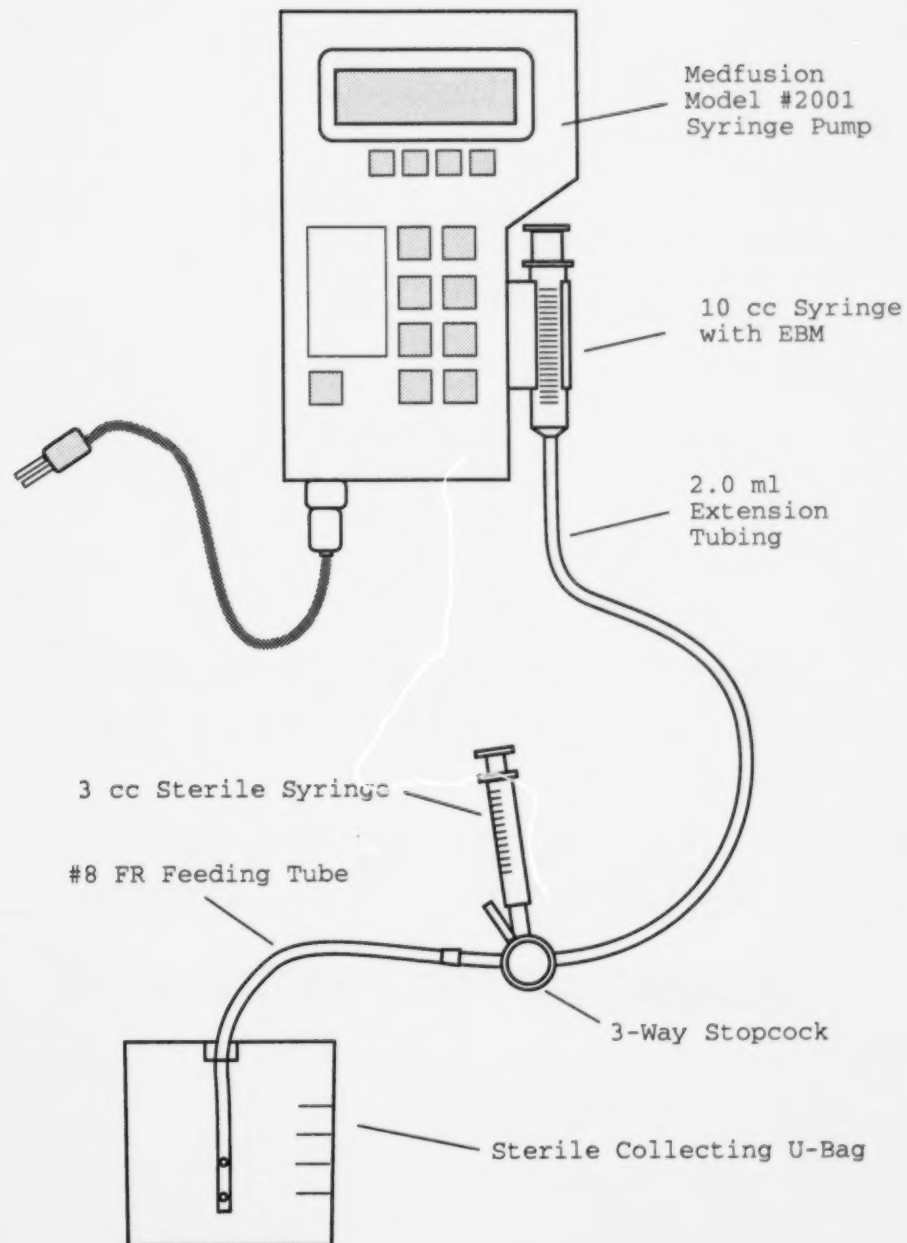


**Figure 1. Simulated Continuous Feeding Setup  
with Expressed Breastmilk.**

## CHAPTER TWO

### LITERATURE REVIEW

The following computerized data bases were searched from 1985 through 1995 (with a few key studies from earlier years), in preparation of this literature review: CINAHL, HEALTHPLAN, MEDLINE, and the OXFORD PERINATAL DATABASE OF TRIALS.

Premature and high-risk neonates are extremely vulnerable to and lack the ability to fight off infections due to an immaturely developed immune system (Bellanti, Boner, & Valletta, 1988; Fanaroff & Martin, 1987). Overwhelming infections can result in serious illness and even death for this population. Consequently, infection control is a critical issue in the NICU.

Both the Committees on Nutrition of the American Academy of Pediatrics and the Canadian Paediatric Society recommend breastmilk to be nutritionally and immunologically the most appropriate milk for infants and to be the food of choice (American Academy of Pediatrics, 1993; American Academy of Pediatrics and American College of Obstetricians and Gynecologists, 1992; Nutrition Committee, Canadian Paediatric Society, 1995; Van Aerde, in press).

Mothers of premature and high-risk neonates are encouraged to express their milk so that it can be fed to their infants when enteral nutrition is commenced, most

often by means of a CFSU. These neonates are thus able to receive all of the immunologic and other benefits of breastmilk despite their inability to breastfeed (American Academy of Pediatrics, 1993; Arnold, 1993; Arnold and Larson, 1993; Garza, Schanler, Butte, & Motil, 1987; Nutrition Committee, Canadian Paediatric Society, 1995).

Both premature and high-risk neonates are at great risk for bacterial infection in the NICU. Infant formula is considered stable for four hours within a CFSU, but little information is known about the stability of EBM in these feeding setups. The American Academy of Pediatrics and the American College of Obstetricians and Gynecologists (1992) have recommended that the syringe and tubing be changed every four hours when infusing EBM in a CFSU, however, no data could be found which substantiated these guidelines. Consequently the question arises as to the length of time that a syringe of EBM can be used to infuse into a CFSU without posing an increased risk of infection to the vulnerable neonate.

Feeding practices and the use of continuous enteral feeding systems (for EBM and formula) can be a potential source of bacterial infection for premature and high-risk neonates. Necrotizing enterocolitis is one such critical infection in this population. Necrotizing enterocolitis in premature and high-risk neonates has been thought to be

linked to enteral feeding. Although the specific pathogenesis of necrotizing enterocolitis is unclear, enteral feeding and bacterial infection are two of the key elements (Kliegman & Walsh, 1992).

This literature review will summarize some of the results reported in the published research related to EBM delivery by continuous feeding setups in the NICU. The key topics addressed include: adult CFSU studies, EBM bacteriology, milk banking, collection, handling and storage of EBM, and NICU CFSU studies.

#### **Bacterial Growth in CFSU in Adults**

Several studies have been conducted in adult patient settings looking at enteral feeding formulas in CFSU and bacterial growth (Anderson, Norris, Godfrey, Avent, & Butterworth, 1984; Anderton, 1985; Belknap-Mickschl, Davidson, Flournoy, & Parker, 1990; Fagerman, Paauw, McCamish, & Dean, 1984; Freedland, Roller, Wolfe, & Flynn, 1989).

Fagerman, Paauw, McCamish, and Dean (1984) investigated the rate of bacterial contamination and the effects of time, temperature and preservative on bacterial growth in adult enteral nutrient solutions. Their findings concluded that there was no significant increase in bacterial contamination following reconstitution using two different settings (pharmacy and dietary department),

bacterial growth at room temperature was not significantly different for three sets of storage conditions (fresh mixing, freezing and refrigeration), bacterial growth increased over time at room temperature and bacterial growth was reduced when potassium sorbate was added to the enteral nutrient solution.

In the adult clinical setting, investigations have been carried out to assess the microbial contamination of continuous drip feedings and the incidence of patient illness (diarrhea and abdominal distention). Belknap-Mickschal, Davidson, Flournoy, and Parker (1990) found a significantly greater incidence of bacterial contamination [ $> 10^3$  colony forming units per millilitre (cfu/ml)] in enteral nutrition that was prepared by dietary staff or nursing staff using tap water and non-aseptic technique when compared to the use of aseptic technique. However, despite the fact that patients who received contaminated formula feedings had a higher incidence of diarrhea, the difference was not statistically significant at  $p = < .05$ .

Amounts of gram negative bacilli in continuous enteral feeding systems have been shown to correlate with frequency of abdominal distention in adult patients (Freedland, Roller, Wolfe, & Flynn, 1989). These authors also found that feedings which did not have to be reconstituted or mixed with any other solution were significantly less

contaminated at 24 hours. These findings are similar to those of Anderson, Norris, Godfrey, Avent, & Butterworth (1984) who found a significant increase ( $> 10^4$  cfu/ml) of bacterial contamination which exceeded public health standards ( $< 2 \times 10^4$  cfu/ml), in formulas that had to be reconstituted or manipulated. Mixing or diluting of feedings appears to pose an increased risk of bacterial contamination to patients.

Commercial enteral nutrient solutions reconstituted with sterile water which have been experimentally contaminated with pathogenic bacteria and cultured over a 24 hour period have shown a significant increase in bacterial growth at room temperature as compared to storage in a 4°C refrigerator (Anderton, 1985). These results affirm that bacterial growth increases over time at room temperature in enteral nutrient solutions.

Other adult studies have been designed to look at patient clinical outcomes and bacterial contamination in relation to the frequency of changing enteral feeding bags and tubing of continuous feeding systems (Elston-Hurdle, Grey, Roy, & Couperus, 1990; Graham, et al., 1993; Grunow, Christenson, & Moutos, 1989). All of these studies reported results that formulas and feeding systems could be used for longer periods of time (24 hours) without causing harm to the adult patient. Emphasis was placed on cleansing of the

enteral feeding setup and formula preparation technique to decrease bacterial colonization.

Although these studies resulted in decreasing adult patient equipment costs and time saving costs for nursing staff, the results cannot be extrapolated to include the NICU patient. The adult immune system is not as susceptible to bacterial infection as is the immune system of the high-risk neonate. As well, all of the adult studies have dealt with commercial formula preparations and not with EBM.

#### **Bacteriology and Human Milk Banking of EBM**

While ready-to-use formula is considered to be sterile, EBM is not. Several studies have shown that EBM is frequently contaminated with non-pathogenic bacteria and rarely ever sterile (Boer, Anido, & Macdonald, 1981; Eidelman & Szilagyi, 1979; El-Mohandes, Schatz, Keiser, & Jackson, 1993; Law, Urias, Lertzman, Robson, & Romance, 1989; Meier & Wilks, 1987; Quinby, Nowak, Lega, & Andrews, 1976; Pittard, Anderson, Cerutti, & Boxerbaum, 1985). Most often the bacteria cultured are normal skin flora (*Staphylococcus epidermidis* and other *Coagulase negative staphylococci*). Some of the pathogenic organisms that have been cultured in varying amounts in EBM include *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus*, *Klebsiella*, *Proteus*, *Acinetobacter*, Group B streptococcus and *Pseudomonas*.

Breastmilk is not routinely screened for bacteria unless there is a concern that an ill infant may have acquired a pathogen from the mother's milk (e.g., *E. coli* meningitis). This was also much more of a concern when NICUs received their EBM supplies from pooled donors from Human Milk Banks. In Canada, Human Milk Banks have all been discontinued except for one in Vancouver, due to the risk of HIV transmission and other infectious agents including CMV, tuberculosis, and Hepatitis B and C. Most of the literature on bacterial growth in EBM is based on Human Milk Bank screenings (Davidson, Poll, & Roberts, 1979; Law, Urias, Lertzman, Robson, & Romance, 1989; Nutrition Committee, Canadian Paediatric Society, 1985; Sauve, Buchan, Clyne, & McIntosh, 1984). The levels of acceptable bacteria in these studies of pooled and unpooled human milk range from  $<10^5$  colony forming units per litre (cfu/L) to  $<10^8$  cfu/L. The absence of pathogenic bacteria in donor milk is also presented as a criterion for human milk banks to follow.

There are no firm criteria for determining the safe level of bacteria in expressed breast milk for premature and high-risk neonates in the NICU. The Nutrition Committee of the Canadian Paediatric Society (1985) recommends that EBM be free of any pathogens and have a bacterial colony count of less than  $10^7$  cfu/L or less than  $10^4$  cfu/ml. Studies in the United States and Britain concur with two similar

requirements for acceptable bacteria criteria in EBM: the absence of any pathogens and bacterial counts ranging from  $<10^3$  to  $10^5$  cfu/ml (Davidson, Poll, & Roberts, 1979; El-Mohandes, Schatz, Keiser, & Jackson, 1993; Meir & Wilks, 1987).

The incidence and amount of gram negative bacilli such as *Escherichia coli* ( $>10^3$  cfu/ml) in human milk continuous feedings have been shown to correlate with clinical findings such as abdominal distention, vomiting, heme positive stools and necrotizing enterocolitis in premature infants (Botsford, Weinstein, Boyer, Nathan, Carman, & Paton, 1986; Carrion & Egan, 1990). These studies show the importance of reducing health risks to premature neonates by ensuring the absence of pathogenic bacteria in EBM.

#### **Collection, Handling, and Storage of EBM**

Bacterial contamination of EBM can occur during collection, handling or storage of the milk (Lowry & Burnsides, 1991; Pardou, Serruys, Mascart-Lemone, Dramaix, & Vis, 1994; Pearse, 1992; Schreiner, Eitzen, Gfell, Kress, Gresham, French, & Moye, 1979). Breast pumps can also be sources of pathogenic bacterial growth leading to contaminated expressed breast milk (Asquith, Pedrotti, Harrod, Stevenson, & Sunshine, 1984; Moloney, Quoraishi, Parry, & Hall, 1987; Wilks & Meier, 1988).

Moloney, Quoraishi, Parry, and Hall (1987)

investigated the source of a *Serratia* outbreak epidemic in a special care baby unit. The investigators found that two of the breast pumps being used for expressing breastmilk were contaminated with the organism that caused the epidemic. This study emphasizes the need for sterile collection equipment and regular bacterial culturing of electric breast pumps that are used by more than one mother.

Most often, contamination occurs during expression (using a manual or electric pump) of the mother's milk as a result of inappropriate cleansing techniques (Costa, 1989; Meier & Wilks, 1987; Wilks & Meier, 1988). There was a significant decrease in the number of bacteria in EBM when mothers were shown a standardized breast cleansing technique using Phisoderm soap (Costa, 1989; Meier & Wilks, 1987). In both studies, the experimental group received guided instructions from a nurse for breast cleansing prior to expressing EBM. These studies support the need for a standardized cleansing technique prior to expressing breastmilk for NICU neonates.

Recent studies have looked at the rate of bacterial contamination in EBM left at room temperature (Ajusi, Onyango, Mutanda, & Wamola, 1989; Barger & Bull, 1987). One study conducted in Kenya, found that it was safe for mothers to express their milk and leave it at room temperature for at least eight hours before unacceptable bacterial levels

occurred (Ajusi, Onyango, Mutanda, & Wamola, 1989). Barger and Bull (1987) found no difference in bacterial growth in EBM stored at room temperature versus EBM stored at 4°C over a ten hour period ( $< 10^4$  cfu/ml). It must be noted that although these studies show that EBM is safe for up to eight hours at room temperature, current guidelines from the American Academy of Pediatrics (1992) suggest that EBM should be refrigerated as soon as possible and any EBM in CFSU should be left for no greater than four hours at room temperature.

A study conducted to assess the amount of bacterial contamination ( $>10^4$  cfu/L) in EBM collected in sterile versus clean containers and utilizing a manual versus mechanical technique, found no significant differences in bacterial colony counts (Pittard, Geddes, Brown, Mintz, & Hulsey, 1991). This study did not however, look at bacterial growth over time based on collection method or container type.

No significant differences were found in bacterial counts between EBM expressed at home or in the hospital by women who used a standardized cleansing technique with plain tapwater (Larson, Zuill, Zier, & Berg 1984). The acceptable bacterial colony count ( $<10^6$  cfu/ml) is however, higher than the acceptable level quoted by the Nutrition Committee of the Canadian Paediatric Society (1985). They also found

that colony counts were not significantly different between the time of expression, 24 hours and 48 hours refrigeration.

Pardou, Serruys, Mascart-Lemone, Dramaix, and Vis (1994) conducted a two part study looking at the effects of refrigeration versus freezing EBM on the following: total IgA, lactose and lipids according to bacterial content at collection time of EBM, how the bacterial flora of human milk changes and bacterial growth changes at room temperature over 6 hours. They found that the immunological properties of EBM are decreased when human milk containing bacteria is frozen resulting in an increase in bacterial counts. Overall bacterial growth increased significantly at room temperature (26°C) in EBM samples which initially had varying amounts of bacteria and were frozen versus refrigerated. A major problem with their study is that they did not identify the specific types of bacteria isolated, that is, pathogenic versus non-pathogenic and where the EBM samples for cultures were obtained (i.e., EBM storage container or feeding setup).

In summary, most of the studies showed that collection, handling and storage of EBM are important procedures to consider when assessing the bacteriological safety of EBM.

#### **EBM in Continuous Feedings**

Premature and high-risk infants in the NICU who cannot

breastfeed receive EBM via an intermittent bolus or continuous infusion method. The latter method is frequently used if an infant is unable to tolerate large amounts of milk at a single feed. The continuous method allows the infant to receive small amounts of milk continuously. Continuous feeds in premature neonates are associated with decreased amounts of respiratory and airflow instability, decreased energy expenditure, and improved weight gain (Blondheim, Abbasi, Fox, & Bhutani, 1993; Grant & Denne, 1991; Heymsfield, Casper, & Grossman, 1987; Toce, Keenan, & Homan, 1987).

Few studies have addressed the issue of bacterial growth in EBM in continuous feedings in the NICU (Botsford, et al., 1986; Dodd & Froman, 1991; Lemons, Miller, Eitzen, Strodbeck, & Lemons, 1983).

Botsford, et al. (1986) studied the amount of bacterial colonization in CFSU infusing fresh and frozen donor EBM, mother's EBM and sterile formula. All types of milk were added to the CFSU every four hours and the tubing was changed every twenty-four hours. The correlation between positive documented sepsis episodes and the amount of bacteria cultured from the CFSU were also examined. Donor milk samples were contaminated more frequently with gram negative bacilli than mothers' own milk samples ( $>10^6$  cfu/ml). Clinically suspected, but not proven, sepsis

(abdominal distention, apnea and bradycardia, necrotizing enterocolitis) was documented in six infants in which the bacteria colony counts of the infusing feedings were  $\geq 10^6$  cfu/ml. Feeding intolerance was documented in neonates whose bacterial cultures revealed a colony count of  $\geq 10^3$  cfu/ml.

Fat content losses, along with bacterial growth in fresh and frozen human milk have been investigated in the past by U.S. researchers, Lemons, Miller, Eitzen, Strodbeck, and Lemons (1983). They found that fresh EBM bacterial growth was significantly greater over time but less than the initial colony counts in frozen samples. Bacterial growth in frozen EBM showed no significant changes in colony counts over an eight hour period, although frozen EBM began with a higher colony count. None of the counts in this study met suggested "safe" bacterial levels by Canadian guidelines ( $< 10^5$  cfu/ml). This may be due to the fact that all samples were collected without the mothers using any type of standardized breast cleansing technique. It should be noted that fat content losses were a separate issue in this study and are not associated with bacterial growth in EBM.

Dodd and Froman (1991) compared the incidence of bacterial growth in EBM and formula in CFSUs of infants in the NICU over a twelve hour period. They found no statistically significant difference in bacterial growth

over time between the two types of feeds. Based on their study findings, the NICU has changed nursing practice by having the nurses change the enteral feeding system and type of milk every eight hours as compared to the previous practice of every four hours. Unfortunately, the small sample size for the EBM group ( $n=5$ ) may have affected their statistical findings. No mention of how often the EBM or formula was added to the CFSU is indicated by the authors.

#### Summary

Feeding of breastmilk to neonates appears to be the most beneficial due to nutritional and immunological properties along with psychological and physical benefits for the mother (Laufer, 1990; Virden, 1988; Wrigley & Hutchison, 1990). A summary of the research literature poses many questions about bacterial growth in EBM and its delivery by CFSU.

Most of the research (Davidson, Poll, & Roberts, 1979; El-Mohandes, Schatz, Keiser, & Jackson, 1993; Meir & Wilks, 1987) has suggested that safe bacterial counts in EBM are in the range of  $<10^3$  to  $<10^5$  cfu/ml or  $<10^7$  cfu/L (Nutrition Committee, Canadian Paediatric Society, 1985). EBM with pathogenic bacteria has been associated with sepsis and feeding intolerance in the neonate. Collection technique, handling, and storage of EBM also contribute to bacterial growth. The frequency of changing a CFSU and of adding EBM

to the setup differs among NICUs. No standard research-based guidelines exist as only a few studies have been conducted which lack validity due to sample size or lack of control for extraneous variables such as consistent method of EBM collection, time of collection, method of obtaining samples for culture, loss of cultures, etc.

It seems apparent that further studies need to be conducted to address the issues of bacterial growth over time in CFSU and the frequency of adding EBM to the system. This current investigation evaluated the efficacy of current nursing practice (related to syringe change frequency of EBM in a CFSU) as well as addressing time and cost issues related to this nursing practice.

# CHAPTER THREE

## METHOD

### Design

A factorial within-groups experimental design (see Table 1) was used to compare the amount of bacterial growth over time between two groups of CFSU simulations (using the same mother's EBM sample). The control CFSU group had syringes of EBM replaced hourly and the experimental CFSU group had syringes of EBM replaced every four hours (see Figure 2). The two by two factorial design that was used was embedded in a pre-post design (Christensen, 1991). The independent variable was time which included the two frequencies of syringe changes (control group = Q1H and experimental group = Q4H) within the 4 times of bacterial cultures from each mother's EBM sample (baseline, 1 hour, 4 hours and post-study). The dependent variable was the amount of bacterial growth in each EBM sample from baseline over the four hour study period in each CFSU.

### Sample

EBM samples were acquired from 45 volunteer breastfeeding mothers of term infants ( $\geq$  37 weeks gestation or post-conceptional age) from the Edmonton Board of Health's "Baby Talk" groups and the University of Alberta Hospitals Level III NICU and Post-partum Units. Data

Table 1

## Factorial Within-groups Experimental Design

| Baseline                                                                                              | Method/Time                                                                                                                                       | 1 Hour                                        | 4 Hours                                        | Post-Study                                                                                                                               |
|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| all 45 samples had a culture sent before being divided to infuse in both CFSU (Q1H and Q4H) infusions | <b>Q1H syringe change setup</b><br><br>(syringes of EBM were changed hourly with EBM that was stored in a 4°C refrigerator during the experiment) | Q1H setup @ 1 hour culture<br><br>45 cultures | Q1H setup @ 4 hour culture<br><br>45 cultures  | all 45 samples of EBM which were kept at 4°C during the experiment, had a post-study culture sent from the bottle the milk was stored in |
|                                                                                                       | <b>Q4H syringe change setup</b><br><br>(a syringe with 4 hours of EBM which was thawed and stored in a 4°C refrigerator was infused over 4 hours) | Q4H setup @ 1 hour culture<br><br>45 cultures | Q4H setup @ 4 hours culture<br><br>45 cultures |                                                                                                                                          |
| <b>45 Cultures</b>                                                                                    |                                                                                                                                                   | <b>90 Cultures</b>                            | <b>90 Cultures</b>                             | <b>45 Cultures</b>                                                                                                                       |

Total EBM Cultures: 270

Note.

Baseline cultures were sent from each EBM sample bottle. All 45 samples of EBM were then divided to infuse through both the Q1H (Control) and Q4H (Experimental) syringe change CFSU simultaneously. Upon completion of the experiment, a post-study culture was drawn from each EBM sample bottle which was stored in a 4°C refrigerator during the study.

## Bacterial Growth 23

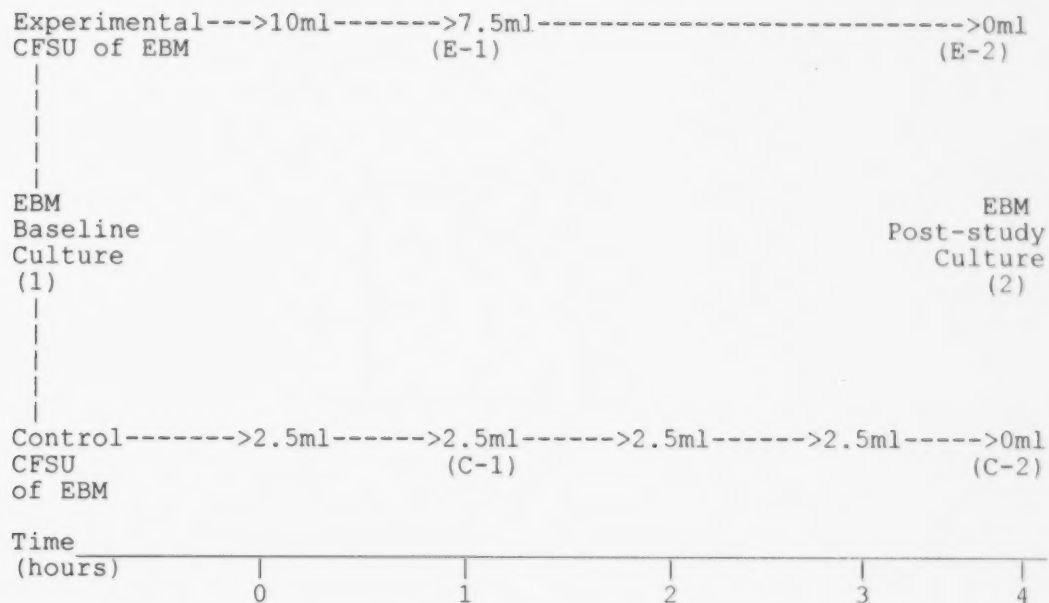


**Figure 2.** Diagram of Experimental Design used for Simulation Experiment and Time of Cultures from EBM Sample.

**Note.**

- Experimental (E) CFSU = 4 hour syringe changes
- Control (C) CFSU = hourly syringe changes
- (1) = Baseline Culture from EBM sample bottle
- (2) = Post-study culture from EBM sample bottle
- C-1 = Control CFSU culture at 1 hour of infusion
- C-2 = Control CFSU culture at 4 hours of infusion
- E-1 = Experimental CFSU culture at 1 hour of infusion
- E-2 = Experimental CFSU culture at 4 hours of infusion

collection for the study was conducted over a four month period from July, 1994 to November, 1994.

Through power analysis, it was determined that a minimum of 44 samples of EBM would be needed for this study with an alpha level set at .05 (Cohen, 1988). Due to some lost data (inadequate EBM sample for testing), the researcher collected and analyzed 45 EBM samples. All EBM samples had a total of 6 analyzed bacterial cultures resulting in 270 quantitative cultures. A large sample size was needed to show any statistical significance in EBM bacterial colony counts if the difference existed. This is attributed to the fact that most studies of EBM at room temperature show that the initial bacteria count is approximately  $10^6$  cfu/L ( $10^3$  cfu/ml) and minimal changes in bacterial counts occur over time.

### **Subjects**

Breastfeeding mothers of term infants ( $\geq$  37 weeks gestation or postconceptional age) were invited to participate in this study after their neonate was greater than ten days of age (to allow the mother to establish breastfeeding and develop a good milk supply). If mothers donated their initial milk, they might have difficulties supplying the EBM sample, as they would not yet have a good milk supply. As well mothers would be donating their colostrum which is high in immunologic factors and essential

for their neonate.

Exclusion criteria for mothers taking part in the study included: cessation of breastfeeding, mothers of premature neonates <37 weeks gestation or post-conceptual age and use of antibiotics  $\leq 48$  hours prior to donating the EBM sample.

Breastfeeding mothers who were on antibiotics or had discontinued their use <48 hours prior to the EBM sample collection were excluded due to the fact that the antibiotics could potentially alter the bacterial colony counts in the EBM.

EBM from mothers of preterm neonates was not used because of the difficulty that most mothers of premature neonates have in expressing milk. As well, since this was a experimental simulation, and all the EBM was discarded at the end, the researcher did not wish to use precious premature mothers' milk for this study.

#### **Ethical Clearance and Considerations**

Ethical clearance for this study was obtained from both the Joint Faculty of Nursing/University of Alberta Hospitals Ethics Review Committee and from the Edmonton Board of Health (EBH). Mothers were invited to participate in this study through the use of a poster (see Appendix A) that was posted in the recruitment areas (NICU, Post-partum Units, and EBH Health Units). Mothers were also recruited

through several "Baby Talk" parent support groups at the Edmonton Board of Health.

Mothers who chose to participate, were contacted by the researcher who explained the research study and obtained informed written consent (see Appendix B). Each participant was given an information sheet about the study (see Appendix C). Mothers were informed that they were free to withdraw from the study at any time. Any mother having difficulty with breastfeeding or infant care concerns was referred to the appropriate health care agency (Paediatrician, Community Health Nurse, Hospital Lactation Consultant). Mothers were also informed that they would be contacted if any pathogenic bacteria were cultured from their breastmilk in the baseline culture.

Anonymity and confidentiality measures were taken. There were no potential risks to mothers who expressed breastmilk for the research study. The research study itself posed no risks as it was a simulation experiment in the NICU environment and no patients were used.

#### **Data Collection**

This research study had two phases. Phase one consisted of EBM sample collection from volunteer breastfeeding mothers (see Appendix D). Phase two was the simulation experiment which took place in the NICU. Each mother's EBM sample was thawed and then divided and infused

through two CFSUs over a four hour period in a simulated NICU environment.

In phase one subjects were instructed by the researcher, who was present for each EBM sample collection, how to cleanse their breasts and express their breastmilk using a standardized technique and a Medela Lactina breastpump or a Medela breastpump (see Appendix D). Each mother used a sterilized Medela breastpump kit and sterile glass bottle which was provided by the researcher, to collect a 45 ml sample of breastmilk. This controlled for any bacteria that could contaminate the EBM sample due to collection technique or equipment.

A total of 45 EBM samples were collected from 45 subjects: 38 EBM samples were collected in subjects' homes by the researcher and 7 samples were collected in the NICU with the researcher present. Collection techniques were similar, except that NICU samples were collected by using the Medela breastpump with universal connecting tubing versus the Medela Lactina breastpump with individual sterilized connecting tubing.

Each EBM sample was labelled with a number, letter, date and time of collection and was stored in a -20°C freezer in the NICU. Immediately upon collection, EBM samples collected outside of the NICU were transported on ice in a plastic cooler to be stored in the -20°C freezer in

the NICU. The rationale for freezing all the EBM samples prior to performing the simulated experiment was to reflect current nursing practice. This is due to the fact that most high-risk neonates cannot tolerate enteral feeding upon admission and consequently EBM is frozen until it can be used for the neonate. Freezing all the EBM samples also enabled the researcher to perform the experiment on each EBM sample consecutively within a short time period.

#### **Research Setting**

The experimental phase of the study was conducted at the Children's Health Centre 30 bed Level III NICU. Although patients were not used for the simulation experiment, the researcher simulated as closely as possible the environment of a neonate in an incubator receiving EBM through a CFSU. Simulating the patient environment allowed the researcher to conduct several continuous feeding setup simulations and obtain bacterial cultures at the same time without having to wait for appropriate subjects.

The study was conducted in an Airshields Isolette Model C-100 with a humidified air temperature set at 36°C. Both the control and experimental CFSUs (Q1H syringe changes and Q4H syringe changes) for each EBM sample infused into the same Airshields Isolette simultaneously over a four hour period. Using the same incubator for the control and experimental CFSU for each EBM sample controlled for any

differences in air temperature or humidity during the experiment. All simulations for the simulation experiment took place over a ten day period in November, 1994.

#### **Research Procedure**

The procedure for the simulated experiment for each mother's EBM sample was identical. Each EBM sample was thawed in lukewarm water (35°C) for a fifteen minute period. This reflects current practice and standard guidelines for thawing EBM (La Leche League International, 1992; Pierce & Tully, 1992; University of Alberta Perinatal Program & Department of Obstetrics and Gynaecology, 1993).

After each EBM sample was thawed, a sample for a baseline bacterial culture was drawn and sent prior to dividing each EBM sample to infuse through the control and experimental CFSUs. Using the same mother's milk simultaneously for both the control and experimental setups controlled for all extraneous variables between the two CFSU except for the frequency of syringe changes of EBM.

A sterile 3cc syringe was used to obtain 0.5cc of EBM for the baseline culture which was placed into a sterile 1.5cc Starstedt Freezer Vial (provided by the Microbiology Lab). This sample was then labelled and sent to the Microbiology Laboratory for quantitative culture analysis. The rationale for the baseline culture from each EBM sample was to determine the bacterial count prior to the start of

the simulation experiment. This controlled for any bacteria or pathogens present prior to beginning the simulation experiment.

Each CFSU consisted of a Medfusion pump Model #2001, 10cc Becton-Dickinson syringe with either 10cc EBM (experimental group) or 2.5cc EBM (control group) infusing at a rate of 2.5cc/hour, 2.0 ml extension tubing set, 3-way stopcock, 3cc syringe for drawing samples for bacterial cultures, #8-FR nasogastric feeding tube and sterile collection U-Bag for collecting the infused milk into. Both the control and experimental CFSU tubing and feeding tubes were suspended 15 cm below the roof of the warmed and humidified Airshields Isolette Model C-100 with an air temperature set at 36°C. Each CFSU had 40 cm of extension tubing outside of the incubator. EBM then infused into the sterile collecting U-Bags during the four hour experiment.

Each EBM sample was then divided to infuse through the two simulated CFSUs (see Figure 1). New CFSUs were used for each EBM simulation. Both the control and experimental CFSU were primed with 10cc of the EBM sample that was being used for the simulation. Once both CFSUs were primed, EBM was added to the setups as follows: 1) the control CFSU had 2.5cc of EBM added hourly (0,1,2,3 hours) with a new sterile 10cc syringe of EBM taken from the EBM sample bottle that was stored in a 4°C refrigerator in the NICU 2) the experimental

CFSU had a 10cc syringe with 10cc of EBM infusing for the four hour period of the study at a rate of 2.5cc per hour. A rate of 2.5cc per hour was chosen to reflect current practice for neonates who are starting continuous feeds with EBM. This rate also allowed the researcher to use only one 10cc syringe of EBM for the experimental CFSU and prevented interruption of the experimental setup.

A sample of 0.5cc of EBM was drawn by the researcher from both CFSUs simultaneously at one and four hours of infusion during the study (see Figure 2). These samples were obtained by drawing EBM with a sterile 3cc syringe at the 3-way stopcock from the 8-Fr nasogastric feeding tube (see Figure 1). The sample was aspirated into the syringe from the direction of the #8-Fr feeding tube but did not include milk that had infused into the sterile collecting U-Bag. Both samples for culture were put into two separate sterile 1.5cc Starstedt freezer vials, labelled with a code number and letter and sent to the Microbiology Lab for analysis. The same method was repeated at 4 hours of EBM infusion for obtaining samples for cultures from both the control and experimental CFSU.

At the end of the study (4 hours), a post-study bacterial culture (using the same technique as was used for the baseline culture) was drawn from the EBM sample bottle that had been stored during the experiment simulation in a

4°C refrigerator in the NICU. The post-study culture provided the researcher with information about the bacterial growth in the EBM that had been stored in the 4°C refrigerator for 4 hours during the experiment. This method was repeated for all forty-five EBM samples.

All EBM culture samples were sent for quantitative analysis to the University of Alberta Hospitals Microbiology Laboratory within 30 minutes of being obtained. The microbiology laboratory technicians were blinded and did not know which EBM sample they were analyzing.

#### **EBM Quantitation by Microbiology Laboratory**

All EBM samples for culture were submitted to the microbiology laboratory in coded and labelled Starstedt freezer vials. Within 30 minutes of specimen collection, 100 microlitres of the sample was inoculated onto a blood agar and MacConkey agar plate. Any remaining EBM specimen was placed in a -40°C freezer for storage until the experimental phase of the study was completed. Agar plates were immediately placed into an incubator and incubated at 35°C for 24 hours.

Plates were assessed for growth by the laboratory staff after the incubation period. If growth was evident, colony counts were determined for each morphotype of bacteria present. All plates were then incubated for an additional 24 hours and colony counts were repeated at this

point. All bacteria present were identified using routine laboratory protocol. Colony counts were then calculated for each morphotype of bacteria present and documented on supplied worksheets with corresponding codes.

Each mother's EBM sample had 6 samples sent for bacterial culture analysis (baseline, 1 hour and 4 hours for both the experimental and control groups, and post-study). The number of EBM quantitative cultures analyzed by the microbiology laboratory were 270 (45 baseline, 90 1-hour cultures, 90 4-hour cultures, and 45 post-study cultures).

#### **Data Analysis**

Data were analyzed using SPSS for Windows (SPSS for Windows-5.0, 1992). The following statistical analyses were used: 1)descriptive statistics, 2)repeated measures analysis of variance (ANOVA), 3)chi-square, and 4)t-test for independent measures.

The microbiology laboratory quantitative culture analysis identified the types of bacteria isolated and the colony counts in cfu/L for each EBM sample at baseline, one hour and four hours of infusion in both CFSU, and post-study. The bacteria colony counts were then transformed to  $10^5$  cfu/L. Transformation of the bacteria counts was performed so that all counts were reported in the same units. Any bacteria colony counts which were  $>10^7$  cfu/L ( $>100 \times 10^5$  cfu/L) were recorded as having a count of "1000"

and were considered to be significant. The presence of pathogenic bacteria was also considered to be significant.

Recoding of the bacteria counts was then carried out in order to perform all the statistical analyses. Bacteria with counts under  $100 \times 10^5$  cfu/L ( $<10^7$  cfu/L) were recoded using the following dichotomous scale:  $0 \times 10^5$  cfu/L = 1,  $1+ \times 10^5$  cfu/L = 2. Bacteria which had colony counts  $>100 \times 10^5$  cfu/L ( $>10^7$  cfu/L) were coded as follows:  $0 \times 10^5$  cfu/L = 1,  $1-999 \times 10^5$  cfu/L = 2,  $1000+ \times 10^5$  cfu/L = 3.

Descriptive statistics were used to report the frequency, range, mean, and standard deviation for the bacteria colony counts of each EBM sample.

Repeated measures ANOVA was used to analyze the amount of bacterial growth over time (using recoded data results) for all subjects using two methods of syringe change frequencies for CFSU. Each mother's EBM sample was its own control. After data results were recoded, there were 45 matched EBM samples measured at 6 different times. The within subject measures for each type of bacteria were the six times that bacterial cultures were drawn: baseline, one hour (control and experimental), four hours (control and experimental), and post-study.

After the bacteria counts were recoded, chi-square analysis was used to analyze the frequencies of the amount of growth of each bacteria over time in the 2 CFSU for each

EBM sample. Chi-square analysis allowed the researcher to compare group frequencies for the 6 time periods that cultures were drawn on each EBM sample. A 2 X 6 chi-square table was used for the analysis. The dependent variable was the amount of bacterial growth which had two categories ( $0-999 \times 10^5$  cfu/L = 1,  $1000+ \times 10^5$  cfu/L = 2). The independent variable was the 6 time periods that bacterial cultures were taken: 1)baseline, 2)one hour of infusion (control CFSU), 3)one hour of infusion (experimental CFSU), 4)four hours of infusion (control CFSU), 5)four hours of infusion (experimental CFSU), and 6)post-study (see Figure 3).

A t-test for independent measures was used to compare the effects of site of EBM sample collection (Home or NICU) on bacteria colony count means (using the recoded data) for the 6 time periods that cultures were drawn for each EBM sample. The t-test for independent measures was used due to the unequal groups where the EBM samples were collected (Home=38, NICU=7).

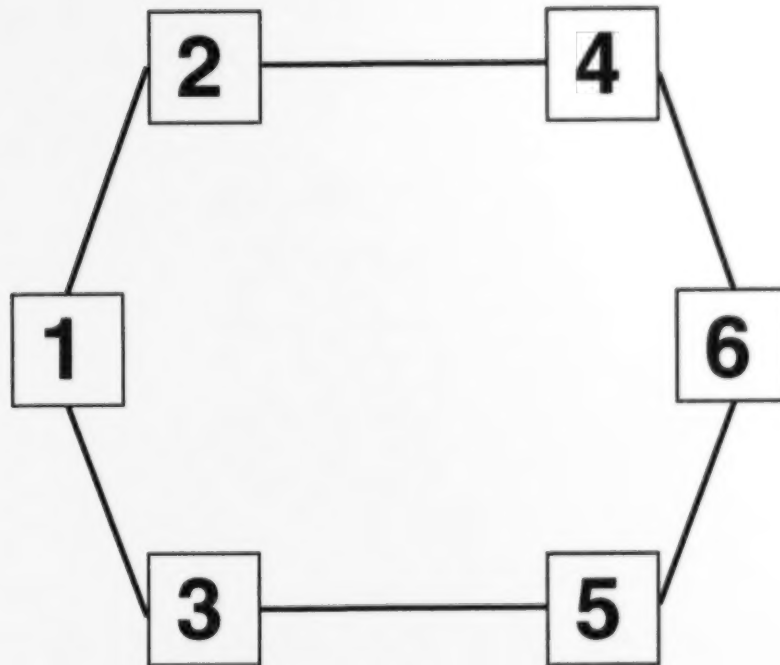


**Figure 3.** Diagram of Control (C) and Experimental (E) Continuous Feeding Setups (CFSU) and Times of Bacterial Cultures.

Note:

- 1 = Baseline culture from EBM sample bottle.
- 2 = Control CFSU culture at 1 hour of infusion.
- 3 = Experimental CFSU culture at 1 hour of infusion.
- 4 = Control CFSU culture at 4 hours of infusion.
- 5 = Experimental CFSU culture at 4 hours of infusion.
- 6 = Post-study culture from EBM sample bottle.

## CHAPTER FOUR

## RESULTS

Forty-five EBM samples (45 ml each) were obtained for the study from 45 volunteer breastfeeding mothers of term infants ( $\geq 37$  weeks gestation or post-conceptual age). Thirty-eight (84.4%) EBM samples were collected in subjects' homes and seven (15.6%) were collected in the NICU. Two additional EBM samples were obtained at home, but were excluded from the study due to inadequate volume.

## Quantitative Culture Analysis

Each of the 45 EBM samples had six samples sent for quantitative culture analysis totalling 269 cultures instead of the planned 270. One culture was not reported due to inadequate volume in the Starstedt vial for analysis by the microbiology laboratory (subject N-6 lacked an adequate amount of EBM sample to be analyzed for culture E1).

Eight different types of bacteria were isolated in the EBM samples through quantitative culture analysis during the simulation experiment (see Table 2). The bacteria colony counts varied between EBM samples and within samples during the simulated experiment. All EBM samples had *Coagulase negative staphylococci*, part of normal skin flora in at least one culture. The non-pathogenic skin bacteria isolated in at least one culture (refer to Appendix E for bar charts) included the following: *Coagulase negative*

Table 2

EBM Sample Quantitative Culture Analysis Results

| Bacteria Isolated                | Mean cfu/L at Time 1  | Mean cfu/L at Time C1 | Mean cfu/L at Time E1 | Mean cfu/L at Time C2 | Mean cfu/L at Time E2 | Mean cfu/L at Time 2  | Type of Bacteria |
|----------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|------------------|
| Coagulase negative staphylococci | 5.03<br>$\times 10^6$ | 5.23<br>$\times 10^6$ | 5.29<br>$\times 10^6$ | 5.32<br>$\times 10^6$ | 6.63<br>$\times 10^6$ | 4.56<br>$\times 10^6$ | NP               |
| Viridans group streptococci      | 8.13<br>$\times 10^5$ | 9.18<br>$\times 10^5$ | 8.36<br>$\times 10^5$ | 1.15<br>$\times 10^6$ | 1.19<br>$\times 10^6$ | 8.73<br>$\times 10^5$ | NP               |
| Diphtheroids                     | 2.6<br>$\times 10^5$  | 1.6<br>$\times 10^5$  | 1.41<br>$\times 10^5$ | 1.76<br>$\times 10^5$ | 1.27<br>$\times 10^5$ | 1.98<br>$\times 10^5$ | NP               |
| Non-pathogenic neisseria species | 3.1<br>$\times 10^4$  | 3.1<br>$\times 10^4$  | 2.0<br>$\times 10^4$  | 4.2<br>$\times 10^4$  | 2.2<br>$\times 10^4$  | 2.7<br>$\times 10^4$  | NP               |
| Staphylococcus aureus            | 1.6<br>$\times 10^4$  | 1.6<br>$\times 10^4$  | 2.0<br>$\times 10^4$  | 2.0<br>$\times 10^4$  | 2.9<br>$\times 10^4$  | 2.2<br>$\times 10^4$  | P                |
| Yeast                            | 0                     | 2.0<br>$\times 10^3$  | 0                     | 7.0<br>$\times 10^3$  | 0                     | 4.0<br>$\times 10^3$  | P                |
| Non-lactose fermenting coliforms | 0                     | 2.0<br>$\times 10^3$  | 2.0<br>$\times 10^3$  | 4.0<br>$\times 10^3$  | 0                     | 0                     | P                |
| Lactose fermenting coliforms     | 0                     | 0                     | 0                     | 0                     | 9.8<br>$\times 10^4$  | 0                     | P                |

Note.

Time 1 = Baseline culture  
 Time C1 = Control CFSU culture at 1 hour  
 Time E1 = Experimental CFSU culture at 1 hour  
 Time C2 = Control CFSU culture at 4 hours  
 Time E2 = Experimental CFSU culture at 4 hours  
 Time 2 = Post-study culture  
 NP = Non-pathogenic  
 P = Pathogenic

*staphylococci* (100%), *Viridans* group streptococci (60%), *Diphtheroids* (35.6%), *Non-pathogenic neisseria species* (13.3%). The pathogenic bacteria isolated in the EBM samples in at least one culture (see Appendix E for bar charts) included the following: *Staphylococcus aureus* (4.4%), *Yeast* (2.2%), *Non-lactose fermenting coliforms* (2.2%), and *Lactose fermenting coliforms* (2.2%). Twenty-one (46.7%) EBM samples had *Coagulase negative staphylococci*  $>10^7$  cfu/L. Only one EBM sample had pathogenic bacteria (*Staphylococcus aureus*) present in the baseline culture (Subject N-3). This subject was notified of the presence of *Staphylococcus aureus* in her EBM, but had discontinued breastfeeding at the time of notification. All other EBM samples contained non-pathogenic bacteria at baseline with one sample (Subject H-1) having no bacteria at baseline.

The means, ranges, and standard deviations were calculated for each bacteria that was isolated in the EBM samples (see Table 3). The non-pathogenic bacteria mean growth ranged from  $2.9 \times 10^4$  cfu/L for *Non-pathogenic neisseria species* to  $5.35 \times 10^6$  cfu/L for *Coagulase negative staphylococci*. Any non-pathogenic bacteria counts that were  $>100 \times 10^5$  cfu/L (same as  $>10^7$  cfu/L) were considered to be clinically significant. This was the case for both *Coagulase negative staphylococci* and *Viridans* group

Table 3

Mean Bacteria Colony Counts in EBM Samples

| Bacteria                                               | Mean Growth<br>(cfu/L) | Standard<br>Deviation<br>(cfu/L) | Range of<br>Growth<br>(cfu/L) |
|--------------------------------------------------------|------------------------|----------------------------------|-------------------------------|
| Coagulase<br>negative<br>staphylo-<br>cocci<br>(Bug 1) | $5.35 \times 10^6$     | $\pm 3.97 \times 10^6$           | 0-> $10^7$                    |
| Viridans<br>group<br>streptococci<br>(Bug 2)           | $9.64 \times 10^5$     | $\pm 2.41 \times 10^6$           | 0-> $10^7$                    |
| Diphtheroids<br>(Bug 3)                                | $1.77 \times 10^5$     | $\pm 5.63 \times 10^5$           | 0- $6 \times 10^6$            |
| Non-<br>pathogenic<br>neisseria<br>species<br>(Bug 4)  | $2.9 \times 10^4$      | $\pm 1.49 \times 10^5$           | 0- $1.3 \times 10^6$          |
| Staphylo-<br>coccus<br>aureus<br>(Bug 5)               | $2.04 \times 10^4$     | $\pm 1.34 \times 10^5$           | 0- $1.3 \times 10^6$          |
| Yeast<br>(Bug 6)                                       | $2.2 \times 10^3$      | $\pm 2.27 \times 10^4$           | 0- $3 \times 10^5$            |
| Non-lactose<br>fermenting<br>coliforms<br>(Bug 7)      | $1.5 \times 10^3$      | $\pm 1.49 \times 10^4$           | 0- $2 \times 10^5$            |
| Lactose<br>fermenting<br>coliforms<br>(Bug 8)          | $1.64 \times 10^4$     | $\pm 2.68 \times 10^5$           | 0- $4.4 \times 10^6$          |

*streptococci*. The pathogenic bacteria (*Staphylococcus aureus*, yeast, non-lactose fermenting coliforms, lactose fermenting coliforms) were not present in significant numbers, but their presence in EBM was still considered to be clinically significant. Mean growth of the pathogenic bacteria ranged from  $1.5 \times 10^3$  cfu/L for *Non-lactose fermenting coliforms* to  $2.04 \times 10^4$  cfu/L for *Staphylococcus aureus*. None of the pathogenic bacteria were present in  $>10^7$  cfu/L in any of the EBM samples.

Yeast (Bug\_6), *Non-lactose fermenting coliforms* (Bug\_7), and *Lactose fermenting coliforms* (Bug\_8) which are considered to be pathogenic, were each present in only 1 EBM sample during the experimental simulation. Due to the fact that these bacteria were present in so few EBM samples and in such insignificant amounts, no further analyses were performed. The remaining bacteria, *Coagulase negative staphylococci* (Bug\_1), *Viridans group streptococci* (Bug\_2), *Diphtheroids* (Bug\_3), *Non-pathogenic neisseria species* (Bug\_4), and *Staphylococcus aureus* (Bug\_5) were present in 2 or more EBM samples in greater amounts and were therefore analyzed using repeated measures ANOVA, chi-square, and t-tests for independent measures. A result of  $p < .05$  was used in all cases to determine a statistically significant result unless otherwise reported.

### Repeated Measures ANOVA Results

Repeated measures ANOVA was used to determine if the amount of bacterial growth increased over time between the 2 CFSUs used in the simulated experiment for the following bacteria: *Coagulase negative staphylococci*, *Viridans group streptococci*, *Diphtheroids*, *Non-pathogenic neisseria species*, and *Staphylococcus aureus*. To perform repeated measures ANOVA, these bacteria were all recoded so that there were 45 matched samples measured on six occasions. The six times that samples for culture were taken for each EBM sample were as follows: time 1 = baseline culture, time 2 = 1 hour culture for control CFSU, time 3 = 1 hour culture for experimental CFSU, time 4 = 4 hour culture for control CFSU, time 5 = 4 hour culture for experimental CFSU, and time 6 = post-study culture (see Figure 3).

Bug\_1 and Bug\_2 had colony counts that were  $>100 \times 10^5$  cfu/L ( $> 10^7$  cfu/L) while Bugs\_3, \_4, and \_5 had colony counts  $<100 \times 10^5$  cfu/L. These bacteria were recoded using a scale of 0-1000+ (see Table 4). The recoded bacteria were used for comparing the 45 matched samples of EBM to see if there was a significant amount of bacteria growing over time between the 2 CFSUs for each subject over the four hour simulated study period.

For analysis of effect of time on bacterial growth repeated measures ANOVA was carried out on the quantitative

Table 4

Recoding of Bacteria for Repeated Measures ANOVA

| Bacteria                                          | Recode Name | Recode Scale<br>(X 10 <sup>5</sup> cfu/L) |
|---------------------------------------------------|-------------|-------------------------------------------|
| Coagulase<br>negative<br>staphylococci<br>(Bug 1) | Bugn11      | 0 = 1<br>1-999 = 2<br>1000+ = 3**         |
| Viridans group<br>streptococci<br>(Bug 2)         | Bugn22      | 0 = 1<br>1-999 = 2<br>1000+ = 3**         |
| Diphtheroids<br>(Bug 3)                           | Bugn33      | 0 = 1<br>1+ = 2                           |
| Non-pathogenic<br>neisseria species<br>(Bug 4)    | Bugn44      | 0 = 1<br>1+ = 2                           |
| Staphylococcus<br>aureus (Bug 5)                  | Bugn55      | 0 = 1<br>1+ = 2                           |

Note. \*\*1000+ = >100 X 10<sup>5</sup> cfu/L (>10<sup>7</sup> cfu/L)

culture analysis for the 45 matched EBM samples measured at 6 times (see Figure 3). The analysis was used to determine if the amount of bacteria growing from time 3 (one hour of infusion, experimental CFSU) to time 5 (four hours of infusion, experimental CFSU) was significantly different from time 2 (one hour of infusion, control CFSU) to time 4 (four hours of infusion, control CFSU). Repeated measures ANOVA, using the recoded data, was also used to compare the amount of bacterial growth between time 1 (baseline culture from EBM sample bottle) and time 6 (post-study culture from EBM sample bottle).

The growth of the following organisms, when subjected to repeated measures ANOVA techniques, proved to not be statistically significant: *Viridans group streptococci*,  $F(5,215) = 0.8134$ ,  $p > .05$  (see Table 6), *Diphtheroids*,  $F(5,215) = 1.4955$ ,  $p > .05$  (see Table 7), *Non-pathogenic neisseria species*,  $F(5,215) = 0.48$ ,  $p > .05$  (see Table 8), and *Staphylococcus aureus*,  $F(5,215) = 1.00$ ,  $p > .05$  (see Table 9). The one result showing statistically significant growth between the 2 CFSU for the 45 subjects was *Coagulase negative staphylococci* (see Table 5),  $F(5,215) = 0.0181$ ,  $p < .05$ , however post-hoc testing using the Tukey HSD Test showed no significant difference between any pair of means ( $p = .14$ ).

Table 5

Repeated Measures ANOVA ResultsCoagulase negative staphylococci (Bugn11) Growth in EBMSamples

| Source of Variation | Sum of SQ | DF  | Mean Square | F    | Prob. |
|---------------------|-----------|-----|-------------|------|-------|
| Between People      | 60.86     | 43  | 1.42        |      |       |
| Within People       | 11.67     | 220 | 0.05        |      |       |
| Between Measure     | 0.71      | 5   | 0.14        | 2.80 | 0.02  |
| Residual            | 10.95     | 215 | 0.05        |      |       |

Table 6

Repeated Measures ANOVA ResultsViridans group streptococci (Bugn22) Growth in EBM Samples

| Source of Variation | Sum of SQ | DF  | Mean Square | F    | Prob. |
|---------------------|-----------|-----|-------------|------|-------|
| Between People      | 66.27     | 43  | 1.54        |      |       |
| Within People       | 23.67     | 220 | 0.11        |      |       |
| Between Measure     | 0.44      | 5   | 0.09        | 0.81 | 0.54  |
| Residual            | 23.23     | 215 | 0.11        |      |       |

Table 7

Repeated Measures ANOVA ResultsDiphtheroids (Bugn33) Growth in EBM Samples

| Source of Variation | Sum of SQ | DF  | Mean Square | F    | Prob. |
|---------------------|-----------|-----|-------------|------|-------|
| Between People      | 32.86     | 43  | 0.76        |      |       |
| Within People       | 11.83     | 220 | 0.05        |      |       |
| Between Measure     | 0.40      | 5   | 0.08        | 1.50 | 0.19  |
| Residual            | 11.44     | 215 | 0.06        |      |       |

Table 8

Repeated Measures ANOVA ResultsNon-pathogenic neisseria species (Bugn44) Growth in EBMSamples

| Source of Variation | Sum of SQ | DF  | Mean Square | F    | Prob. |
|---------------------|-----------|-----|-------------|------|-------|
| Between People      | 10.07     | 43  | 0.23        |      |       |
| Within People       | 5.83      | 220 | 0.03        |      |       |
| Between Measure     | 0.06      | 5   | 0.01        | 0.48 | 0.78  |
| Residual            | 5.77      | 215 | 0.03        |      |       |

Table 9

Repeated Measures ANOVA ResultsStaphylococcus aureus (Bugn55) Growth in EBM Samples

| Source of Variation | Sum of SQ | DF  | Mean Square | F    | Prob. |
|---------------------|-----------|-----|-------------|------|-------|
| Between People      | 6.42      | 43  | 0.15        |      |       |
| Within People       | 1.33      | 220 | 0.01        |      |       |
| Between Measure     | 0.03      | 5   | 0.01        | 1.00 | 0.42  |
| Residual            | 1.30      | 215 | 0.01        |      |       |

### Chi-Square Analysis

Further analyses using chi-square with a 2 X 6 table (2 = 2 bacterial growth frequencies and 6 = 6 times of bacterial cultures) was carried out to determine any difference in the amount of bacterial growth over time using the 2 CFSU for the 45 matched EBM samples.

Results of chi-square analyses showed that the amount of bacterial growth of *Coagulase negative staphylococci*, *Viridans group streptococci*, *Diphtheroids*, *Non-pathogenic neisseria species*, and *Staphylococcus aureus* in the EBM samples did not reach statistical significance,  $p > .05$  (see Table 10). This confirmed that the bacterial growth frequencies over time between the 2 CFSUs for each EBM sample did not differ in statistical significance.

### Independent Measures t-test Results

An independent measures t-test was used to analyze the effect of site of EBM collection (Home or NICU) on the amount of bacterial growth over the six time periods at which cultures were drawn for each EBM sample. There was no effect on the amount of bacterial growth of *Viridans group streptococci*, *Non-pathogenic neisseria species*, or *Staphylococcus aureus* in EBM samples collected at home or in the NICU (see Tables 11-13).

*Coagulase negative staphylococci* bacteria growth was statistically significant ( $p < .05$ ) in the EBM samples that

Table 10

Chi-Square Analysis Results for Amount of Bacterial Growth  
by Bacteria Type

| Bacteria                                           | Value | DF | Significance | p value |
|----------------------------------------------------|-------|----|--------------|---------|
| Coagulase<br>negative<br>staphylococci<br>(Bugn11) | 2.34  | 5  | .80          | p > .05 |
| Viridans group<br>streptococci<br>(Bugn22)         | 1.04  | 5  | .96          | p > .05 |
| Diphtheroids<br>(Bugnn33)                          | 2.21  | 5  | .82          | p > .05 |
| Non-pathogenic<br>neisseria<br>species<br>(Bugn44) | 1.07  | 5  | .96          | p > .05 |
| Staphylococcus<br>aureus<br>(Bugn55)               | 1.01  | 5  | .96          | p > .05 |

Table 11

Independent Measures t-test Results on Site of EBMCollection (Home or NICU)Viridans group streptococci

| Culture Time      | t Value | DF | 2-Tail Sig | p value |
|-------------------|---------|----|------------|---------|
| 1<br>(Baseline)   | -0.08   | 43 | .934       | p > .05 |
| 2<br>(1hr C)      | -0.29   | 43 | .772       | p > .05 |
| 3<br>(1hr E)      | 0.44    | 43 | .663       | p > .05 |
| 4<br>(4hrs C)     | 0.51    | 43 | .613       | p > .05 |
| 5<br>(4hrs E)     | 0.17    | 43 | .863       | p > .05 |
| 6<br>(Post-study) | 0.88    | 43 | .382       | p > .05 |

\* = value statistically significant

\*\*C = Control CFSU

\*\*E = Experimental CFSU

Table 12

Independent Measures t-test Results on Site of EBM Collection(Home or NICU)Non-pathogenic neisseria species

| Culture Time      | t Value | DF   | 2-Tail Sig | p value |
|-------------------|---------|------|------------|---------|
| 1<br>(Baseline)   | -0.80   | 6.41 | .451       | p > .05 |
| 2<br>(1hr C)      | -0.54   | 43   | .595       | p > .05 |
| 3<br>(1hr E)      | -1.02   | 42   | .314       | p > .05 |
| 4<br>(4hrs C)     | 0.76    | 43   | .453       | p > .05 |
| 5<br>(4hrs E)     | 0.61    | 43   | .545       | p > .05 |
| 6<br>(Post-study) | 0.76    | 43   | .453       | p > .05 |

\* = value statistically significant

\*\*C = Control CFSU

\*\*E = Experimental CFSU

Table 13

Independent Measures t-test Results on Site of EBM Collection(Home or NICU)Staphylococcus aureus

| Culture Time      | t Value | DF | 2-Tail Sig | p value |
|-------------------|---------|----|------------|---------|
| 1<br>(Baseline)   | -1.00   | 6  | .356       | p > .05 |
| 2<br>(1hr C)      | -1.00   | 6  | .356       | p > .05 |
| 3<br>(1hr E)      | -1.00   | 5  | .363       | p > .05 |
| 4<br>(4hrs C)     | -1.55   | 6  | .172       | p > .05 |
| 5<br>(4hrs E)     | -1.00   | 6  | .356       | p > .05 |
| 6<br>(Post-study) | -1.55   | 6  | .172       | p > .05 |

\* = value statistically significant

\*\*C = Control CFSU

\*\*E = Experimental CFSU

were collected in the NICU as compared to those collected in the home (see Table 14). *Diphtheroids* growth in EBM samples collected at home was also statistically significant ( $p < .01$ ) as compared to EBM samples collected in the NICU (see Table 15).

A statistically significant difference was found in the growth of *Coagulase negative staphylococci*,  $t(43) = -2.57$ ,  $p = .014$  and *Diphtheroids*,  $t(37) = 4.13$ ,  $p = .000$  in the baseline cultures of EBM samples collected in the NICU. *Coagulase negative staphylococci* growth in EBM samples collected in the NICU differed significantly from Home collected samples at all times that cultures were drawn except for the four hour culture in the experimental CFSU,  $t(43) = -1.71$ ,  $p = .094$ .

The growth of *Diphtheroids* bacteria in EBM samples collected at home differed significantly for the following times: experimental CFSU at one hour of infusion,  $t(37) = 2.89$ ,  $p = .006$ ; control CFSU at four hours of infusion,  $t(37) = 3.64$ ,  $p = .001$ ; experimental CFSU at four hours of infusion,  $t(37) = 3.14$ ,  $p = .003$ .

The mean bacterial colony counts of each bacteria, based on site of EBM sample collection (Home or NICU) were computed (see Table 16). This was done to determine if the statistically significant results for *Coagulase negative staphylococci* and *Diphtheroids* were due to a possible outlier effect. The results

Table 14

Independent Measures t-test Results on Site of EBM Collection(Home or NICU)Coagulase negative staphylococci

| Culture Time      | t Value | DF | 2-Tail Sig | p value |
|-------------------|---------|----|------------|---------|
| 1<br>(Baseline)   | -2.57   | 43 | .014*      | p < .05 |
| 2<br>(1hr C)      | -2.57   | 43 | .014*      | p < .05 |
| 3<br>(1hr E)      | -2.95   | 42 | .005*      | p < .01 |
| 4<br>(4hrs C)     | -2.19   | 43 | .034*      | p < .05 |
| 5<br>(4hrs E)     | -1.71   | 43 | .094       | p > .05 |
| 6<br>(Post-study) | -2.74   | 43 | .009*      | p < .01 |

\* = value statistically significant

\*\*C = Control CFSU

\*\*E = Experimental CFSU

Table 15

Independent Measures t-test Results on Site of EBM Collection(Home or NICU)Diphtheroids

| Culture Time      | t Value | DF | 2-Tail Sig | p value |
|-------------------|---------|----|------------|---------|
| 1<br>(Baseline)   | 4.13    | 37 | .000*      | p < .01 |
| 2<br>(1hr C)      | 0.40    | 43 | .689       | p > .05 |
| 3<br>(1hr E)      | 2.89    | 37 | .006*      | p < .01 |
| 4<br>(4hrs C)     | 3.64    | 37 | .001*      | p < .01 |
| 5<br>(4hrs E)     | 3.14    | 37 | .003*      | p < .01 |
| 6<br>(Post-study) | .67     | 43 | .507       | p > .05 |

\* = value statistically significant

\*\*C = Control CFSU

\*\*E = Experimental CFSU

Table 16

Mean Bacterial Colony Counts Based on Site of EBM SampleCollection (Home or NICU)

| Bacteria Type                    | Mean Growth in cfu/L (Home) | SD of Growth in cfu/L (Home) | Mean Growth in cfu/L (NICU) | SD of Growth in cfu/L (NICU) |
|----------------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|
| Coagulase negative staphylococci | $4.8 \times 10^6$           | $\pm 3.9 \times 10^6$        | $8.2 \times 10^6$           | $\pm 3.3 \times 10^6$        |
| Diphtheroids                     | $2.1 \times 10^5$           | $\pm 6.1 \times 10^5$        | $4.9 \times 10^3$           | $\pm 2.2 \times 10^4$        |
| Viridans group streptococci      | $1.0 \times 10^6$           | $\pm 2.5 \times 10^6$        | $7.8 \times 10^5$           | $\pm 1.5 \times 10^6$        |
| Non-pathogenic neisseria species | $3.3 \times 10^4$           | $\pm 1.6 \times 10^5$        | $9.8 \times 10^3$           | $\pm 3.7 \times 10^4$        |
| Staphylococcus aureus            | no growth                   | no growth                    | $1.3 \times 10^5$           | $\pm 3.2 \times 10^5$        |
| Yeast                            | $2.6 \times 10^3$           | $\pm 2.5 \times 10^4$        | no growth                   | no growth                    |
| Non-lactose fermenting coliforms | $1.8 \times 10^3$           | $\pm 1.6 \times 10^4$        | no growth                   | no growth                    |
| Lactose fermenting coliforms     | no growth                   | no growth                    | $1.1 \times 10^5$           | $\pm 6.9 \times 10^5$        |

of this analysis showed that there wasn't an outlier effect for *Coagulase negative staphylococci* growth in the NICU samples. Sixty (26%) cultures of the Home collected samples had a *Coagulase negative staphylococci* colony count  $>10^7$  cfu/L, compared to 30 (73%) cultures from the NICU collected samples.

There was an outlier effect detected for *Diphtheroids* growth in the Home samples accounting for the significant differences based on site of EBM sample collection. One hundred and seventy-three cultures (76%) of the Home collected EBM samples had no growth of *Diphtheroids* bacteria and only one culture had a count of  $6 \times 10^6$  cfu/L. In the NICU collected samples, 39 (95%) of the cultures had no growth of *Diphtheroids* bacteria with only 2 (5%) cultures having  $1.0 \times 10^5$  cfu/L. When the outlier effect was removed there was no significant difference in *Diphtheroids* growth between the two sites of EBM sample collection.

## CHAPTER FIVE

### DISCUSSION

The results of this study show that there was no statistically significant difference ( $p > .05$ ) in the amount of bacterial growth in EBM infused over a four hour period between the 2 CFSUs (one hour syringe changes compared to four hour syringe changes) in a simulated NICU environment. There was, however a statistically significant difference on the amount of *Coagulase negative staphylococci* bacteria growing in the EBM samples collected in the NICU ( $p < .05$ ) compared to EBM samples collected at home.

#### EBM in Continuous Feedings

This study showed no significant differences in the amount of bacterial growth in EBM in the control and experimental CFSU over a four hour period at room temperature. The results of this study concur with those of Lemons, Miller, Eitzen, Strodbeck, and Lemons (1983), Dodd and Froman (1991), and Pardou, Serruys, Mascart-Lemone, Dramaix, and Vis (1994) with respect to bacterial growth in previously frozen EBM in CFSU.

Lemons, Miller, Eitzen, Strodbeck, and Lemons (1983) performed a simulated experiment with fresh and frozen EBM using a CFSU setup similar to that used in this study. Although Lemons, Miller, Eitzen, Strodbeck, and Lemons (1983) looked at bacterial counts in both fresh and frozen

milk over an 8 hour period with cultures done at 0, 5, 6, 7, and 8 hours of infusion, they did not perform cultures at baseline (before infusing the EBM through the CFSU) and post-study. Despite having a higher mean bacteria colony count at time 0 ( $5.0 \times 10^7$  cfu/L) than that found in this study ( $2.03 \times 10^5$  cfu/L), the overall differences in bacterial growth over an 8 hour period were not statistically significant ( $p > .05$ ). Interestingly, CFSU infusing fresh EBM, had lower mean bacteria counts at time 0, but by 6 hours of infusion, there was a statistically significant ( $p < .05$ ) increase in bacterial growth. They recommended that frozen milk infuse in a CFSU for only 4 hours and that the complete CFSU and syringe of EBM be changed after 4 hours due to the statistically significant caloric loss of the EBM beyond the 4 hour period.

The findings of Pardou, Surreys, Mascart-Lemone, Dramaix, and Vis (1994) also suggest that EBM syringes be changed every 4 hours for EBM that has been frozen due to the fact that freezing does not inhibit the development of bacteria. Their findings indicated that frozen EBM with low initial non-pathogenic bacteria counts ( $9.5 \times 10^5$  cfu/L) do not increase significantly for even up to 6 hours of infusion in a CFSU. On the contrary, frozen EBM samples with pathogenic bacteria counts which are initially higher ( $4.90 \times 10^6$  cfu/L) do not increase significantly at 4 hours

of infusion at room temperature, while at 6 hours of infusion the bacteria do increase significantly ( $p = .05$ ).

Dodd and Froman (1991) found that bacterial growth in EBM which had been frozen and infused at room temperature, did not significantly increase ( $p > .05$ ) over a 12 hour study period in CFSU in the NICU. Their results however, only assessed for growth of the following bacteria: *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterobacteriaceae*, and *Escherichia coli*, and an "other category" which was not specified. The number of EBM samples in this study were only 5, which reflects a very small sample size. As well the initial mean non-pathogenic bacteria counts in Dodd and Froman's (1991) study were higher ( $4 \times 10^7$  cfu/L) than those which were found in the current study ( $2.02 \times 10^5$  cfu/L) and no comment was made on the pathogenic bacteria counts. The current study isolated a greater number of non-pathogenic bacteria species than Dodd and Froman (1991), as well as isolating Yeast, Lactose fermenting coliforms, and Non-lactose fermenting coliforms.

Overall the main issue in the current study was to determine if the amount of bacteria in EBM increased significantly over a four hour period in a CFSU. The lack of significant growth over a time period of 4 hours is heartening. The findings of this study supports the practice of infusing breastmilk for a 4 hour period without

risk of increased bacterial growth and possible potential harm to the neonate.

It must be kept in mind, however that this was a simulation study not using live patients. As a result, these findings can only be applied to the effect of syringe change frequency in CFSU infusing EBM. Further studies using patients need to be conducted assessing the effect of bacterial growth on CFSU which are changed every 24 hours, but EBM is added in new syringes every 4 hours. The safety of this practice for vulnerable neonates could then be addressed.

This study did not measure fat content loss in EBM infusing in a CFSU and therefore no comments about that issue can be made. But based on Lemons, Miller, Eitzen, Strodbeck, and Lemons (1983) findings and those of Brennan-Behm, Carlson, Meier, and Engstrom (1994) assessing caloric loss from EBM in CFSU to be significant ( $p < .025$ ), this is an important issue to keep in mind when infusing EBM in CFSU. Brennan-Behm, Carlson, Meier, and Engstrom (1994) found that the loss of milk lipids from EBM infusing in CFSU ranged from 2.32 Kcal/oz to 2.77 Kcal/oz when comparing microbore tubing and standard bore tubing.

Caloric loss from EBM due to adherence of the milk lipids to the CFSU tubing means fewer calories for the vulnerable growing premature neonate. This is a concern as

the premature and high-risk neonate require these calories for growth.

#### Quantitative Culture Analysis

The results from the quantitative culture analysis of this study concurs with previous studies (Boer, Anido, & Macdonald, 1981; Eidelman, & Szilagyi, 1979; El-Mohandes, Schatz, Keiser, & Jackson, 1993) that EBM is rarely ever sterile and usually contains varying amounts of non-pathogenic bacteria. The non-pathogenic skin bacteria (*Coagulase negative staphylococci*, *Viridans group streptococci*, *Diphtheroids*, and *Non-pathogenic neisseria species*) found in the EBM samples of this study were similar to those found in other studies. Specifically, *Coagulase negative staphylococci* was the predominant organism cultured from EBM samples in this study (100%).

Pittard, Anderson, Cerutti, and Boxerbaum (1985) isolated *Coagulase negative staphylococci* 100% of the time in EBM samples while El-Mohandes, Schatz, Keiser, and Jackson (1993) found that 76% of their EBM samples had *Coagulase negative staphylococci*. Similarly Boer, Anido, and Macdonald (1981) cultured between 94-97% *Coagulase negative staphylococci* in their EBM samples depending on their patient population (private versus non-private). *Coagulase negative staphylococci* and *Streptococcus viridans* were isolated in 82% of EBM samples obtained from mothers

using a method of collection in which there was no contact with the skin of the areola (Carrol, Davies, Osman, and McNeish, 1979).

Meier and Wilks (1987) also found that *Staphylococcus epidermidis* (a *Coagulase negative staphylococci* and part of normal skin flora), contaminated 100% of EBM cultures in their study in which mothers used a similar cleansing technique to the one used for this study.

Growth of the non-pathogenic bacteria in this study ranged from  $1.3 \times 10^6$  cfu/L for *Non-pathogenic neisseria species* to  $>10^7$  cfu/L for *Coagulase negative staphylococci* and *Viridans group streptococci*. Meier and Wilks (1987), in whose study a similar cleansing technique was used for collecting EBM, had an even greater range of results with  $10^5$  cfu/L to  $10^9$  cfu/L of non-pathogenic bacteria growth in their samples. This study may in fact have had similar upper limits of growth, but the laboratory reported all high colony counts simply as being  $>10^7$  cfu/L. The reason for this was the cost of doing further analysis and the cut off for acceptable non-pathogenic bacterial growth was pre-selected by the researcher to be  $\leq 10^7$  cfu/L as per the Canadian Paediatric Society's guidelines for bacteria in EBM (Nutrition Committee, Canadian Paediatric Society, 1985). Consequently, the researcher did not request counts for growth higher than the acceptable levels.

Twenty-one (46.7%) of the 45 EBM samples obtained for this study showed *Coagulase negative staphylococci* growth  $>10^7$  cfu/L. Most studies state that acceptable levels of non-pathogenic bacteria in EBM should be  $<10^7$  cfu/L (El-Mohandes, Schatz, Keiser, & Jackson, 1993; Meier & Wilks, 1987; Nutrition Committee, Canadian Paediatric Society, 1985). Despite these cut-off levels, there really is limited corresponding clinical data to indicate that EBM with these non-pathogenic bacteria colony counts is unsafe for high-risk or premature neonates. After all, the non-pathogenic bacteria isolated in this study were all normal skin flora which cannot be eliminated from the breast. Even utilizing a specialized and stringent cleansing technique with the researcher present and supervising at each EBM sample collection did not eliminate normal skin flora, except for one EBM sample (Subject H-1). The researcher thus questions the clinical significance of these guidelines for non-pathogenic bacteria in EBM samples.

The pathogenic organisms which were isolated in the EBM samples in at least one culture included: *Staphylococcus aureus*, *Lactose fermenting coliforms*, and *Non-lactose fermenting coliforms*. Yeast was also isolated in one EBM sample. Most often the pathogenic bacteria which have been isolated in other studies include: *Staphylococcus aureus*, *Klebsiella*, *Enterococcus*, *Group B streptococcus*, *Escherichia*

*coli*, and *Pseudomonas*.

EBM studies analyzing bacterial growth indicate that the presence of pathogenic bacteria in EBM is unacceptable for consumption by neonates in the NICU and may be related to negative clinical findings in these neonates (Meier and Wilks, 1987; Moloney, Quoraishi, Parry, & Hall, 1987).

Only one EBM sample (Subject N-3) had any pathogenic bacteria (*Staphylococcus aureus*) present in the baseline culture (this subject was contacted and notified of the findings, but had discontinued breastfeeding at the time of notification). The remaining pathogenic bacteria were isolated during the simulated study, although there is a question as to whether or not they could have been present in the baseline culture, but were not isolated by the microbiology laboratory. They could also have been possible contaminants during the study (despite efforts to prevent this from occurring).

*Staphylococcus aureus* was isolated in at least one culture of two mothers' samples (4.4%) which were collected in the NICU and in none of the EBM samples collected in the homes of subjects. Similar findings by El-Mohandes, Schatz, Keiser, & Jackson (1993), Meier and Wilks (1987), and Sauve, Buchan, Clyne, & McIntosh (1984) indicate that the frequency of *Staphylococcus aureus* growth in EBM samples ranged from 0.9% to 12.5% (the site of collection for the EBM specimens

in these studies was not always indicated).

*Staphylococcus aureus* in EBM is considered pathogenic, however it could be a contaminant during the EBM collection for the study, although strict measures were taken to prevent this by using sterilized breast pumps and a cleansing technique. Two other explanations for the presence of *Staphylococcus aureus* in the EBM samples could be maternal skin colonization by this organism, or colonization or contamination of the "universal" breastpump tubing used in the NICU.

Up to 25% of adults can be colonized with *Staphylococcus aureus* and become prolonged or intermittent carriers of this bacteria with a carrier rate of 20-40% (Waldvogel, 1995). The two EBM samples which had this bacteria isolated may be due to the fact that these mothers may be colonized with *Staphylococcus aureus* and are thus carriers of this bacteria.

Another explanation could be the "universal" breast pump tubing which is utilized in the NICU for the Medela Breast Pump between each mother (tubing and air filter are not changed between mothers). Mothers who participated in this study from the NICU used both a sterile Medela breast pump kit, but did not use sterile connecting tubing. Subsequently, mothers who had their milk collected in their homes used a sterile Medela breast pump kit and connecting

tubing.

Moloney, Quoraishi, Parry, and Hall (1987) performed a study which cultured various parts of 12 electric breast pumps including the air filters and connecting tubing and found that several pathogenic organisms including *Staphylococcus aureus*, *Klebsiella* and *Serratia marcescens* were isolated. An outbreak of *Serratia* in their nursery was connected to the use of the electric breast pumps by the mothers of babies who had the organisms. This reinforces the need for mothers to each use their own connecting tubing and air filters when expressing breastmilk in the NICU.

Both *Lactose fermenting coliforms* and *Non-lactose fermenting coliforms* are pathogenic bacteria, but were each isolated in only one EBM sample (2.2%) from two different mothers. An explanation for this may be contamination of the subject's hands, despite handwashing. Another explanation might be collection equipment contamination or study procedure contamination.

Yeast in EBM is actually not considered to be pathogenic and was most likely isolated due to maternal breast colonization. Only one EBM sample from one mother (2.2%) had Yeast isolated in this study. This mother's breasts may have been colonized with yeast if the infant had oral thrush at the time of the EBM sample collection. At the time the EBM sample was collected, the subject did not

indicate that she or her infant may have had a yeast infection.

#### Site of EBM Collection

The amount of *Coagulase negative staphylococci* and *Diphtheroids* bacterial growth in EBM samples during the simulated experiment was found to be statistically significant ( $p < .05$ ) based on site of collection (Home or NICU). Further analysis looking at the mean growth of these bacteria based on site of collection revealed that *Diphtheroids* growth in the Home samples had an outlier effect which accounted for the significant difference. As a result, the *Diphtheroids* growth in home collected samples was not statistically significant. Although the bacteria are non-pathogenic, the fact that where the EBM sample was collected affected the bacterial growth warrants further investigation.

EBM samples which were collected in the NICU had statistically significant ( $p < .01$  and  $< .05$ ) higher amounts of *Coagulase negative staphylococci* growth for all study times except the 4 hour experimental study CFSU culture. Once again this could be due to the universal pump tubing used for the EBM collection in the NICU.

The results of this study regarding site of EBM collection contradicts those of Larson, Zuill, Zier, and Berg (1984) who found no significant difference in the

amount of bacterial colonization in EBM expressed at home compared to EBM expressed in the NICU using a standardized cleansing technique. It would be beneficial to conduct further studies looking at the effect of site of EBM collection using a larger sample size with an equal number of subjects both from home and in the NICU.

#### **Limitations of the Study**

Some of the limitations to this study include the following: sample size, simulated environment, equipment, design, and instrumentation.

#### **Sample Size**

Although power testing revealed that a sample size of 44 would be adequate to show a medium effect size with an alpha level of .05, an even stronger power could have been achieved by having a larger sample size. The cost of a larger sample size is an issue because of expenses involved with the microbiology laboratory doing bacterial quantitative culture analysis. As well, the chance of a Type II error (failing to reject a false null hypothesis) would be decreased with a larger sample size.

Sample size is also an issue with respect to the results of the presence of *Staphylococcus aureus* in the EBM samples. The NICU sample was small and comments about the presence of this bacteria is limited. A larger sample size with an equal amount of NICU and home collected samples

would be needed to show any significance.

The loss of data for this study was minimal. One culture from the control CFSU was not analyzed due to inadequate EBM sample, while minimal, it is still lost data which may have affected the results for the control group.

#### Simulated Environment

The results of this study cannot be generalized to support the safety of this practice for neonates in the clinical setting due to the simulated environment which was used. What can be said about this study is that the efficacy of changing syringes of EBM was analyzed and results indicate that bacterial growth of previously frozen EBM does not increase significantly over a 4 hour period regardless of syringe change frequency at room temperature.

To analyze the safety of this nursing practice for vulnerable neonates, a study using the same method as described for this study needs to be conducted in the NICU environment using patients. Using real patients will also mean that the effects of an indwelling feeding tube would also be addressed.

Temperature variations in the NICU were not addressed by this study. Although this study was conducted in an isolette with a set air temperature, neonates also receive EBM in CFSU in open cots in our NICU.

### Equipment

This study controlled for various extraneous variables such as: environment temperature, breast cleansing method, numerous personnel involvement, and method of expressing breastmilk. However, one difference between NICU and home collected EBM samples was the use of the "universal" breast pump connecting tubing in the NICU (home subjects used individual sterilized connecting tubing). This could account for the presence of *Staphylococcus aureus* and significant increases in *Coagulase negative staphylococci* growth in the NICU collected EBM samples.

It has been well documented by Moloney, Quoraishi, Parry, and Hall (1987) that breast pumps and breast pump attachments such as the connecting tubing are areas of transmission of bacteria between mothers using the same pump. Further studies which compare the use of both universal connecting tubing and sterile tubing for breast pumps and which analyze bacterial growth are indicated.

### Design

The design of this study could be improved by having equal NICU and home collected EBM samples (although each subject's EBM sample in this study served as her own control). The design could also be improved by conducting this experiment over a 24 hour period with cultures being drawn at one and four hours of infusion every four hours.

In this way, the effects of the CFSU infusion tubing and feeding tube on bacterial growth could be determined. Due to increased costs involved in having such a large number of cultures analyzed, this was beyond the scope of the current study.

#### Instrumentation

All samples for culture were analyzed quantitatively by the microbiology laboratory in an accredited, licensed facility. There is always the slim possibility of human error with this method of analysis. As well it would have been beneficial to perform a further breakdown of the *coagulase negative staphylococci* into specific types (e.g. *staphylococcus epidermidis*) and specific colony counts, rather than just reporting results as  $>10^7$  cfu/L.

#### **Nursing Practice Implications**

Although this study was conducted in a simulated NICU patient environment, based on this study, current nursing practice for syringe change frequency of EBM in a CFSU can be addressed. Syringes of previously frozen EBM can be changed every four hours without an increased risk of bacterial growth in the CFSU used in the NICU. Adopting this practice will result in decreasing nursing time with respect to the previous labour intensive hourly syringe changes of EBM. This will also result in less frequent disturbances of vulnerable high-risk and premature neonates.

The end result is a potential cost-savings of approximately \$10,000.00 per year on syringes alone (based on approximately 200 premature and high-risk neonates receiving EBM through a CFSU for a three week period with syringe cost-savings calculated a \$2.41 per day). The savings on nursing time is approximately 2 hours per twelve hour shift (based on 10 minutes per hour) which results in 4 hours per day. Based on the average registered nurse's salary at \$21.27 per hour, the salary savings amounts to \$31,054.20 per year. This salary saving on nursing time can be used for providing other more meaningful aspects of nursing care to these neonates. In these times of health care reform and cutbacks, these are important issues to be considered.

#### **Conclusion**

Utilizing the continuous infusion method for delivering EBM to high risk and premature neonates is frequently the method of choice for neonates who cannot tolerate large volumes of feeds. The assumption that there is an increased risk of bacterial contamination of the infusing EBM over time has been a concern.

This simulated research study evaluated the amount of bacterial growth in EBM in CFSU over a four hour period comparing two frequencies of syringe changes (one hour compared to four hours). Results indicated that there is no

significant difference in the amount of bacterial growth in the CFSU regardless of whether syringes of EBM are changed hourly or every four hours. These results concur with other studies (Dodd & Froman, 1991; Lemons, Miller, Eitzen, Strodbeck, & Miller, 1983; Pardou, Derruys, Mascart-Lemone, Dramaix, & Vis, 1994) which found that bacterial growth in previously frozen EBM did not significantly increase over time in CFSU. These results substantiate the American Academy of Pediatrics guidelines for allowing EBM to infuse over a four hour period (American Pediatric Society and the American College of Obstetricians and Gynecologists, 1992).

The results of this research study will also decrease the amount of nursing time needed for changing syringes of EBM on an hourly basis (approximately 10 minutes per hour to get the milk from the refrigerator and change the syringe of EBM , which amounts to 4 hours per day). Being able to change syringes of EBM every four hours will allow the nurse to have more time to provide other more meaningful aspects of nursing care to these vulnerable neonates. The potential cost-savings of \$10,000.00 per year on syringes and \$31,000.00 per year on nursing time are also positive results of this research study.

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**APPENDIX A**

Poster for Subject Recruitment

Appendix A

Poster for Subject Recruitment

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Breastfeeding Mothers  
of  
Full Term Babies

=====

**WE NEED YOUR HELP**

**TO TAKE PART IN A STUDY THAT IS  
LOOKING AT HOW LONG PUMPED  
BREASTMILK CAN STAY AT ROOM  
TEMPERATURE IN THE NURSERY  
BEFORE IT SPOILS**

We need mothers who are breastfeeding to volunteer to give a small amount (1 1/2 ounces) of pumped breastmilk. We will show you how to pump your milk with a breast pump. The nurse researcher will come to your home with the breast pump and will pick up the milk. This will only take about 30 minutes of your time.

**If you are interested in being in this study,  
please call:**

Christene Evanochko-Henwick RNC, BSN  
Masters of Nursing Student  
Faculty of Nursing  
492-9167

Dr. M.R. Elliott PhD, RN  
Professor  
Faculty of Nursing  
492-6241

Bacterial Growth 88

**APPENDIX B**

Consent Form

**Appendix B**

INFORMED CONSENT FORM

Project Title

Bacterial Growth in Expressed Breastmilk in Continuous Feeding  
Setups in the Neonatal Intensive Care Unit (NICU)

Investigator

Christene Evanochko-Henwick RNC,BSN  
Graduate Student  
Faculty of Nursing  
University of Alberta  
Office Phone: 492-9167

Faculty Advisor

Dr. M. Ruth Elliott PhD, RN  
Professor  
Faculty of Nursing  
University of Alberta  
Phone: 492-6241

Purpose of the Study

The purpose of this study is to find out how long a mother's breast milk can stay at room temperature in a special feeding setup in the nursery before it spoils.

Procedure

You will be asked to pump 1 1/2 ounces of breast milk into a special bottle with a number on it. The bottle and cleaning supplies will be given to you by the researcher. The researcher will teach you how to clean your breasts and how to express the milk into the bottle. After the milk has been collected, please put the bottle of milk into your own or the hospital's freezer and tell the researcher. The researcher will pick the milk up and take it on ice to the NICU. The milk will be kept in a special freezer until it is used for the study. You will be notified if your breastmilk has any uncommon bacteria in it.

Participation

Participation in this study is voluntary. There is no risk to you or your baby, if you take part in this study. All information will be used so that you cannot be identified. You will not benefit directly from this study. Results from this study may help nurses in the NICU find out how often expressed breast milk should be added to the special feeding setups that are used. This may help to improve the type of care that nurses give to babies who cannot breast feed and receive breast milk through a special feeding setup. You do not have to be in this study if you do not want to be. If you choose to be in the study, you may still drop out at any time by telling the researcher. You will not have to tell anyone why you decided to drop out. You do not have to answer any

questions. Taking part in this study or dropping out of this study will not affect you or your baby's care in the hospital.

Confidentiality

Neither your name nor your baby's name will appear in this study. This form will be kept in a locked drawer during the study and for five years after the study is complete. All of the research data will be stored in a locked cabinet, separate from the consent form, for a minimum of seven years. The results may be used for another study in the future, if the researcher receives approval from the appropriate ethical review committee. The information and findings of this study may be published or presented at conferences, but your name or any material that may identify you or your baby will not be used. If you have questions or concerns about this study at any time, you can call the researcher at 492-9167.

Consent

This is to certify that I, \_\_\_\_\_ (print name) hereby agree to participate as a volunteer in the above named research study. I have had a chance to ask whatever questions I have about this study and my part in it and I am satisfied with the answers. I am aware that records of this study will be kept private. I understand that there are no benefits or risks to me or my baby by taking part in this study. I understand that I am free to drop out of the study at any time and nothing will happen to me or my baby. I have been given a copy of this form to keep. I know that I may phone the researcher named above, at 492-9167 if I have any questions either now or later.

\_\_\_\_\_  
(Signature of Mother)

\_\_\_\_\_  
(Date)

\_\_\_\_\_  
(Phone Number)

\_\_\_\_\_  
(Signature of Researcher)

\_\_\_\_\_  
(Date)

-----  
If you wish to receive a summary of the study when it is finished, please complete the following:

Name: \_\_\_\_\_

Address: \_\_\_\_\_

Postal Code: \_\_\_\_\_

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**APPENDIX C**

Information Sheet

**Appendix C**

Information Sheet

Breast Feeding Mothers of Full Term Babies

I am doing a study to look at the amount of time that pumped breastmilk can stay at room temperature in a special feeding setup before it spoils. This feeding setup is used to give breastmilk to sick and premature babies in the intensive care nursery who cannot breastfeed.

If you participate in this study, you will be asked to donate 1 1/2 ounces of breastmilk. I will provide you with an electric breast pump to pump your milk into a special bottle (provided by me). I will show you how to cleanse your breasts and pump your breastmilk using this pump. This one time donation would take about 30 minutes of your time. I will ask you to do this once only.

Taking part in this study is totally voluntary. No harm will come to you or your baby if you choose not to take part in this study.

If you are interested in taking part in this study, your name will be given to me. I will then contact you and discuss the details of the study with you.

**Investigator**

Christene Evanochko-Henwick RNC, BSN  
Masters in Nursing Student  
Faculty of Nursing  
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Office Phone: 492-9167

**Faculty Advisor**

Dr. M.R. Elliott PhD, RN  
Professor  
Faculty of Nursing  
University of Alberta  
Office Phone: 492-6241

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**APPENDIX D**

Instruction Sheet

Appendix D

Mother's Instructions for Expressing Breast Milk

- 1) Wash hands thoroughly with soap and water. Clean under and around fingernails.
  - 2) Arrange sterile milk collection equipment beside pump. Do not touch the inside of the collecting bottle or the bottom of the lid.
  - 3) Hand express and discard the first 2 teaspoons (10 cc) of milk from each breast.
  - 4) Cleanse your nipples and areolae as follows:  
(repeat for both breasts)
    - \*wet 4 sterile gauze wipes with sterile water
    - \*begin cleaning with nipple and work outward in a circular fashion until the areola is cleaned
    - \*rinse nipples and areola with 2 wet sterile gauze pads
    - \*dry nipples and areola with dry sterile gauze pads
  - 5) Place the collecting device over one breast and begin to use the pump. Take care not to touch the inside of the pumping device or your nipple area.
  - 6) When the breast is emptied, pour your milk into the sterile bottle that the researcher has given you. Do not touch the inside of the sterile bottle or the lid (Repeat steps #5 and #6 with your other breast until you collect a total of 1 1/2 ounces).
  - 7) Date and time the sterile numbered bottle of breast milk and notify the researcher. Do not put your baby's name on the bottle. The bottle has a special number on it for the researcher. Place the bottle of milk in your freezer or the hospital's freezer as soon as you finish pumping your milk.
- \*\*the sterile water, sterile gauze pads and sterile numbered bottle will be supplied by the researcher

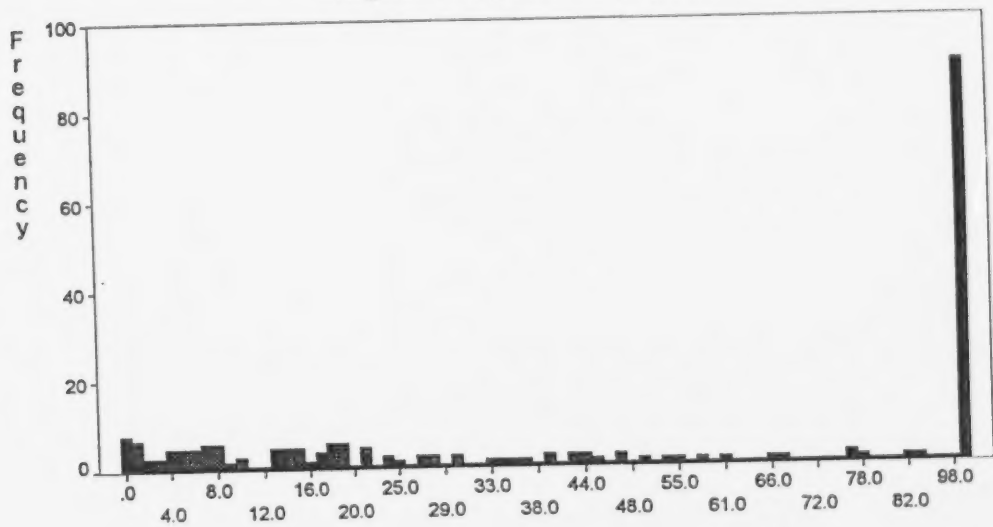
(adapted from: Meier, P., & Wilks, S. [1987]. The bacteria in expressed mothers' milk. Maternal-Child Nursing Journal, 12, pp.421.)

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**APPENDIX E**

Bar Charts of Bacterial Growth in EBM

Appendix E1  
Bar Chart for Bacterial Growth in EBM Samples  
Coagulase negative staphylococci



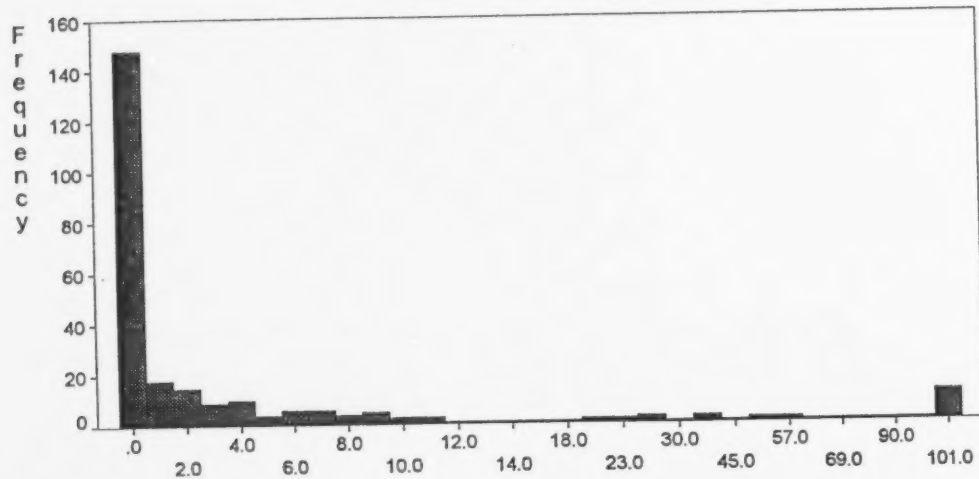
Coagulase negative staphylococci X 10<sup>-5</sup> cfu/L

Coagulase negative staphylococci growth in EBM samples from 269 cultures

## Appendix E2

### Bar Chart for Bacterial Growth in EBM Samples

Viridans group streptococci



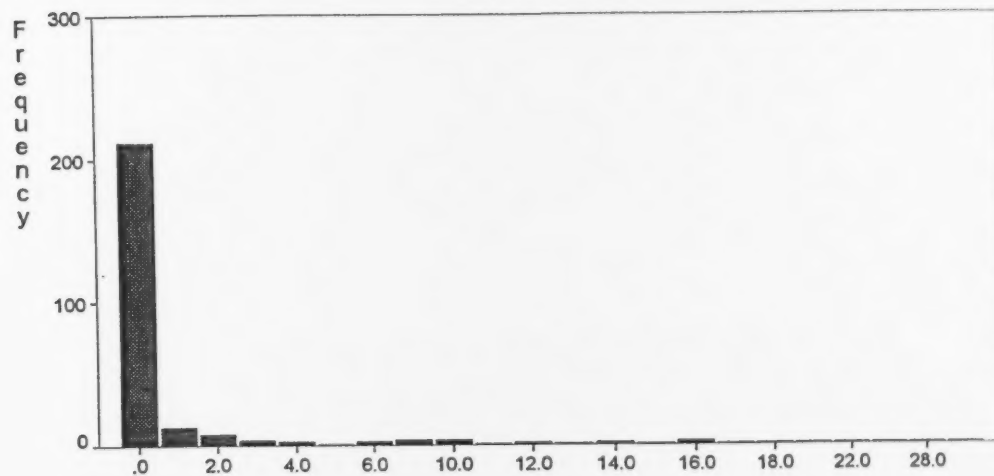
Viridans group streptococci X 10<sup>-5</sup> cfu/L

Viridans group streptococci growth in EBM samples from 269 cultures

### Appendix E3

#### Bar Chart for Bacterial Growth in EBM Samples

##### Diphtheroids



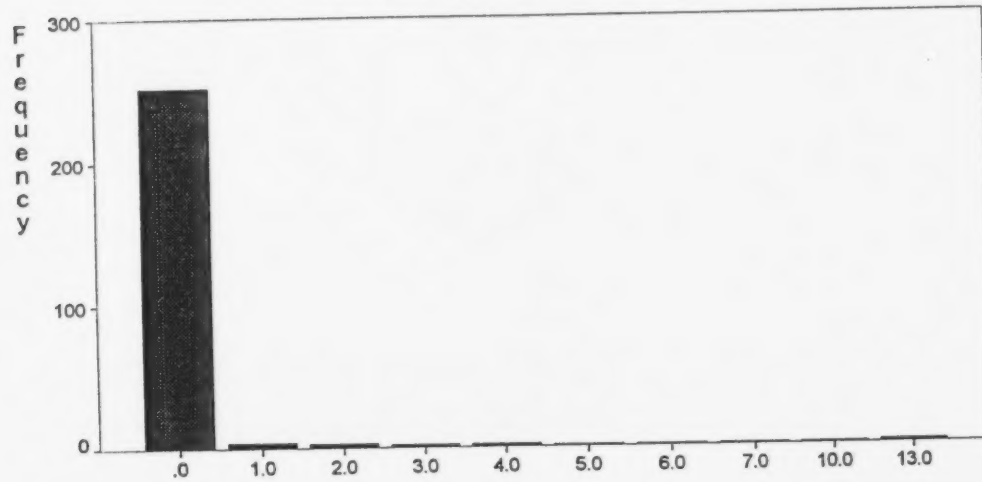
Diphtheroids X 10-5 cfu/L

Diphtheroids growth in EBM samples from 269 cultures

## Appendix E4

### Bar Chart for Bacterial Growth in EBM Samples

Non-pathogenic neisseria species



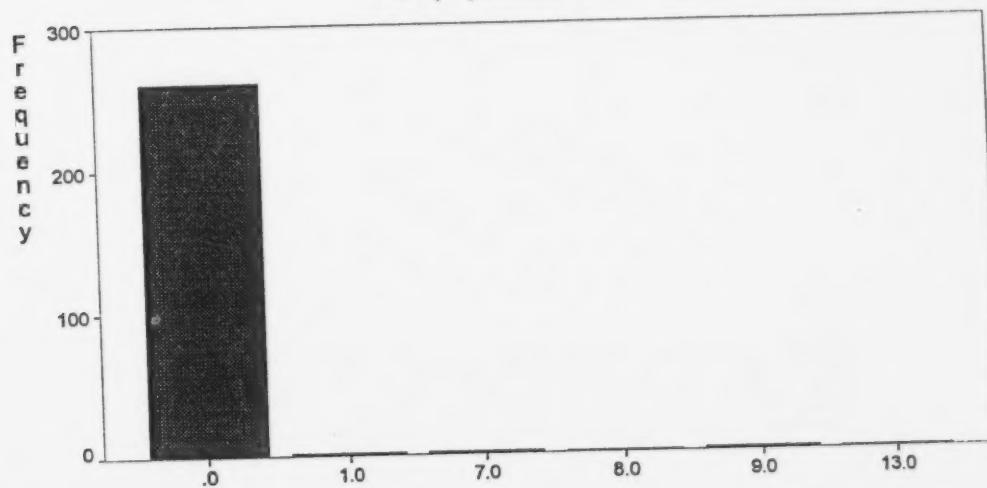
Non-pathogenic neisseria species X 10<sup>-5</sup> cfu/L

Non-pathogenic neisseria species growth in EBM samples from 269 cultures

## Appendix E5

### Bar Chart for Bacterial Growth in EBM Samples

*Staphylococcus aureus*



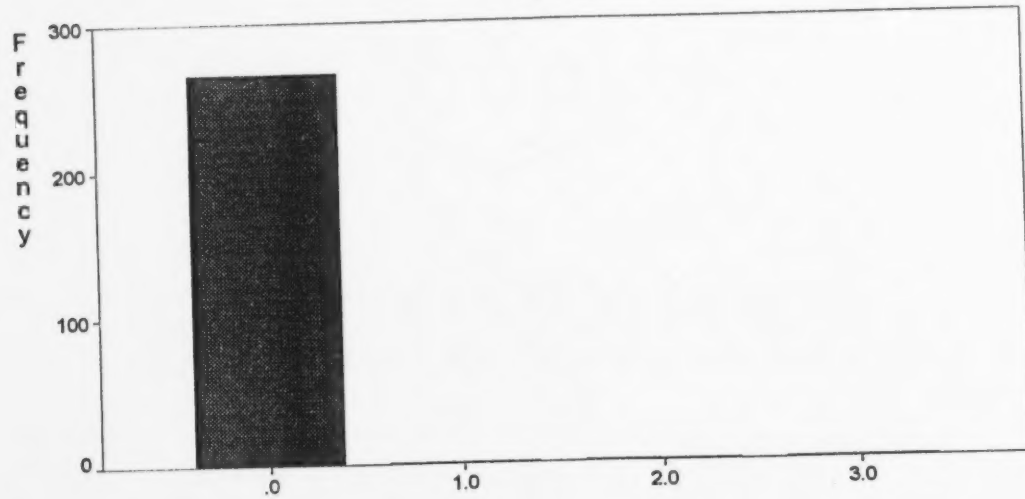
Staphylococcus aureus X 10-5 cfu/L

Staphylococcus aureus growth in EBM samples from 269 cultures

Appendix E6

Bar Chart for Bacterial Growth in EBM Samples

Yeast



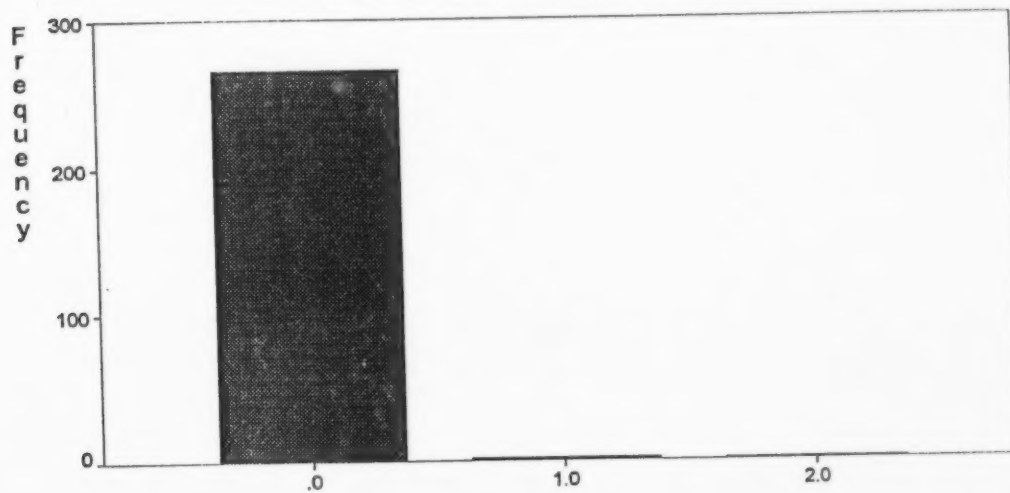
Yeast X 10-5 cfu/L

Yeast growth in EBM samples from 269 cultures

### Appendix E7

#### Bar Chart for Bacterial Growth in EBM Samples

Non-lactose fermenting coliforms



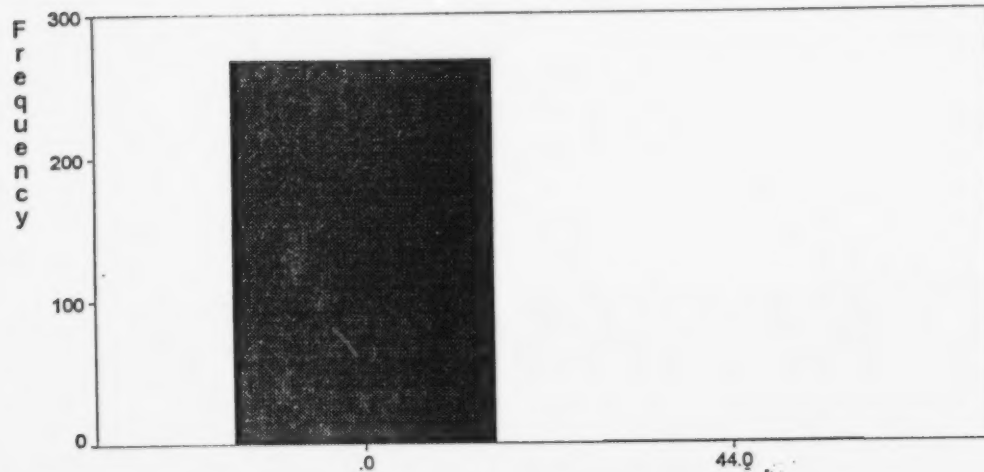
Non-lactose fermenting coliforms X 10-5 cfu/L

Non-lactose fermenting coliforms growth in EBM samples from 269 cultures

## Appendix E8

### Bar Chart for Bacterial Growth in EBM Samples

Lactose fermenting coliforms



Lactose fermenting coliforms X 10<sup>-5</sup> cfu/L

Lactose fermenting coliforms growth in EBM samples from 269 cultures